RELATIONSHIPS BETWEEN DISEASE, WORK & NUTRITION IN DRAUGHT CATTLE & BUFFALO

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Declaration

I Richard Guy Clemence declare that this thesis has been composed by myself and that the work described herein was conducted by myself except where otherwise indicated.

Signed

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ABSTRACT

It is well known that disease adversely affects the production of milk and meat in domestic livestock and therefore it would be surprising if certain diseases did not also affect the work output of draught animals. There is however no direct evidence of this, although there is strong evidence from research with humans and laboratory animals that exercise depresses immune responses. This effect may be exacerbated by undernutrition. Draught animals are required to work hard for short periods during the year, these periods often coincide with seasonal food shortages and sometimes with outbreaks of disease. The combination of work, disease and malnutrition may have serious adverse effects on the ability of draught animals to complete cultivation tasks. In countries with short rainy seasons this can have a potentially disastrous effect on subsequent crop yields.

The interactions of disease, work and undernutrition in draught animals were examined in three separate studies. The first study carried out in Indonesia investigated the effects of *Trypanosoma evansi* on the work output of twelve swamp buffalo in two groups. The second undertaken in the UK, used four groups of six sheep as a model to examine the effects of exercise and plane of nutrition on cellular and humoral immune responses in the absence of a pathogen. The final study in The Gambia, looked at the effects of work and undernutrition on the trypanotolerance of 32 N'Dama cattle in four groups challenged with *T. congolense*. In the first experiment work output was monitored for 11 weeks in infected and uninfected animals using a cross-over design. In the second laboratory based experiment the immune responses of sheep challenged with two foreign antigens *Brucella abortus* and ovalbumin, were measured for 11 weeks after challenge in a factorial design involving two planes of nutrition and two levels of exercise. In the third study the same factorial design was used to examine the effects of work and undernutrition on the trypanotolerance of N'Dama cattle challenged with *T. congolense*. The results of

all three studies were analysed using either parametric and non-parametric statistical tests as appropriate.

In the first experiment carried out in Indonesia, after a protracted period of work (5 weeks pre-infection and 4 weeks post-infection), the parasitaemias of some infected buffalo increased dramatically and at the same time work output declined. However because of the experimental design and because a similar effect was not seen in the first period of the experiment, it was not possible to prove that trypanosomosis had caused this fall in output. In the second study with sheep, immune responses varied markedly between individuals but there were no significant differences between groups, with one exception, the speed of the primary antibody response to ovalbumin was significantly faster in sheep on the high plane of nutrition than in those on the low plane. In the final study in The Gambia work caused significant increases in animal parasitaemias and reductions in blood packed cell volumes. Some of the working cattle became so severely anaemic (PVC's < 15%) that they were unable to complete the normal daily work programme and had to be retired early, the first after only seven weeks work.

In conclusion it appears that in some circumstances work can affect the course of a disease and conversely disease can reduce work output. It is however very difficult to quantify these effects because of large differences in response between individual animals and differences in pathogenicity between different diseases and different strains of the same disease.

Contents

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List of abbreviations

Organisations

CTVM	Centre for Tropical Veterinary Medicine, University of Edinburgh, Scotland.
DAFS	Department of Agriculture & Fisheries for Scotland
ITC	International Trypanotolerance Centre, Banjul, The Gambia.
MAFF	Ministry of Agriculture Food & Fisheries, UK Government.
NRI	Natural Resources Institute, UK Government.
ODA	Overseas Development Administration, UK Government.
RIVS	Research Institute for Veterinary Science, Bogor, Indonesia.

General Abbreviations

ADF	Average draught force
CNS	Central nervous system
DG	Dark ground illumination technique, for quantifying trypanosome parasitaemias.
EDTA	Ethylene diamine tetra acetic acid
FACS	Fluorescence activated cll sorting
GE	Gross energy
i.m.	intra-muscular
i.v.	intra-venous
K_m	Efficiency of utilisation of metabolisable energy to supply net energy
LEV	Log equivalent value
LRS	Lymphoreticular system
LW	liveweight
$LW^{0.75}$	metabolic liveweight
ME_{maint}	Metabolisable energy required for maintenance
MHCT	Microhaematocrit centrifugation technique for detection of trypanosome infections in blood samples.
NE	Net energy

OD	Optical density
PBS	Phosphate buffered saline
PCV	Packed red blood cell volume
p.i.	post-infection
PSG	Phosphate saline glucose
q_m	metabolisability
RBC	Red blood cell count
s.c.	sub-cutaneous
TMB	Tetramethylbenzidine dihydrochloride
WBC	White blood cell count

Units

°C	degree centigrade
dl	(1×10 litres)
fl	(1×10^{-15} litres)
g	gravitational units
h	hour
i.u.	international units
J	joule
kg	kilogram (1×10^{3}g)
mg	milligrams (1×10^{-3}g)
ml	millilitre (1×10^{-3} litres)
µl	microlitre (1×10^{-6} litres)
m	metre
mm	millimetre (1×10^{-3} metres)
nm	nanometre (1×10^{-9} metres)
min	minute
N	Newton
pg	picograms (1×10^{-12}g)
s	second
W	watt

Statistics

ANOVA	Analysis of variance
CL	Confidence limit
ems	Error mean square
KW	Kruskal Wallis test
LSD	Least significant difference
MW	Mann Whitney test
P	Probability
R	Correlation coefficient
s.e.	Standard error
s.e.d.	Standard error of the difference
s.i.r.	Semi inter-quartile range

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1. INTRODUCTION

For thousands of years draught animals have been the only alternative to manual labour in agriculture world-wide. Over the last century the internal combustion engine has replaced the horse and the ox on farms in the developed world. At the beginning of the 1970's when oil was cheap, tractors looked set to replace draught animals in all but the most inaccessible fields in developing countries too, as farmers and governments aspired to modern technology. To date this has not happened. Oil prices have risen dramatically, while the purchase and maintenance costs of tractors have remained beyond the reach of many farmers with small land holdings. Today some 400 million animals continue to provide a vital source of power in developing countries, enabling farmers to cultivate larger areas more intensively than they would otherwise be able to do with human labour alone. In some parts of the world draught animal power is used alongside human labour, in others it is integrated with more mechanised forms of power, with tractors doing the primary cultivation and animals providing the power for weeding and harvesting. Furthermore, there remain large areas of land in Asia and elsewhere where draught animals provide the only viable alternative to hand labour for reasons of accessibility or because of operating conditions, such as the terraces of Nepal or the rice paddies of S.E. Asia. There is no reason to believe that this will change markedly in the foreseeable future.

One of the oft cited advantages of draught animal power as compared to mechanical power is that draught animals don't break down requiring expensive spare parts to repair them and that furthermore draught animals will reproduce and replace themselves, before providing a valuable source of food at the end of their working lives. Whilst this is true draught animals do get lame, sick and on occasion die. There

is also much speculation supported by anecdotal and circumstantial evidence that sub-clinical disease in particular, may be considerably reducing the work output of draught animals on many farms world-wide (Hoffman and Dalgliesh, 1985; Payne, Djauhari, Partoutomo, Jones and Pearson, 1991; Wells, 1986). Partoutomo, Ronohardjo, Wilson and Stevenson (1985), noted the effects of such diseases are not dramatic and consequently they may remain undetected for considerable periods of time. Some scientists working in Asia have observed reductions in work output in buffalo which could be attributed to disease (Pearson, 1989b; Roberts, Bakrie, Copeman and Teleni, Unpublished; Rukmana, 1979). Similarly reports from Africa suggest the productivity of working cattle is compromised by disease (Anon., 1985; Bourn and Scott, 1978; Samui and Hugh-Jones, 1990). In 1986 Wells commented that there was very little quantitative data available on the effect of infections on the working abilities of draught animals, the situation has not improved over the last 10 years. In 1993, Dharsana and Campbell stated that there was an urgent need for more studies on the interactions of work and disease.

A fall in the work output of draught animals due to infection or any other cause, can lead to poor crop yields particularly when growing seasons are short, and consequently to lower overall farm productivity. The only alternatives available to a farmer in this position are to reduce the area cultivated or keep more draught animals, both of which will also have a detrimental effect on the productivity of the farm.

If diseased animals work less well than healthy ones, conversely does work exacerbate the course of a disease? There is evidence that work may increase the susceptibility of draught animals to disease (Munzinger, 1982; Starkey, 1981). Following a survey of farmers in Java, Indonesia, Kenyon, Fauzi, Burton and Darsana (1989), concluded that working animals appeared to be more susceptible to common diseases than non-working animals on the same farms. In humans there is evidence that severe or acute exercise increases susceptibility to disease (Fitzgerald, 1991).

Work may have an important effect on the pathogenicity of disease through its action as a stressor affecting immunological responses. Most of the studies on the effects of exercise on immune responses in animals have been performed in laboratory animals and have involved very short bouts of exercise rather than prolonged work over several weeks or months such as would be experienced by draught animals in the field.

If work does exacerbate disease, then the worsening disease may in turn further depress work output, until the afflicted animal is unable to work at all or dies. The incapacity or death of a draught animal during the cultivation season is a major catastrophe for an owner who often cannot afford to keep or hire a replacement. Furthermore rearing and training a replacement may take several years; good, trained animals are seldom sold.

At the start of the working season draught animals are often in very poor condition, following under-nutrition during the dry season, which led Wells (1986) to speculate that poor body condition combined with the stress of work may make such animals even more vulnerable to disease. Malnutrition is known to impair immune function (Kelley, 1980; Tizard, 1992), which in turn can increase susceptibility to infection and the pathogenicity of the subsequent disease.

In many tropical farming systems cultivation operations must be carried out promptly at the start of the rains to allow the growing crops to make maximum use of soil moisture. Thus draught animals are often worked hardest when feed resources are scarce and when their fitness is low, at a time when environmental conditions are often right for the rapid replication and spread of disease pathogens. This combination of stressors was cited as the possible cause of an increase in the incidence of haemorrhagic septicaemia in working cattle and buffalo in Asia (Centre for Tropical Veterinary Medicine, 1976) and an upsurge in clinical trypanosomosis in draught buffalo in N.Vietnam after the Vietnam war (Wells 1982).

Disease, work and nutrition in draught animals appear to interact in a multifactorial feedback system, each parameter affecting one or both of the other two, which may in turn feedback to the primary parameter. The relationships are complex and not yet well understood. The work detailed in this thesis was undertaken to explore these relationships more fully. The aims of the work were:

1. To examine the effects of infection on the work performance of draught animals and quantify any changes in work output under controlled experimental conditions using a pathogen prevalent in draught animals. To achieve this a study was carried out at the Research Institute for Veterinary Science (RIVS) in Bogor, W. Java, Indonesia which measured the work performance of swamp buffalo with and without *Trypanosoma evansi* infections.

2. To evaluate techniques for investigating immune responses in working animals in the field. Two techniques for assessing humoral and cellular immune response in working animals were tested in an experiment at the Centre for Tropical Veterinary Medicine (CTVM) in Edinburgh, using sheep inoculated with non-pathogenic antigens as a model for large ruminants with pathogenic infections.

3. To investigate the interactions of disease, work and nutrition in animals worked at a similar intensity to farmers' animals. This was done by artificially infecting trypanotolerant N'Dama cattle on two planes of nutrition with *T. congolense* and monitoring their subsequent immune responses, work performance and disease resistance. Their performance was compared to that of unworked but infected animals on the same two planes of nutrition. This final study was carried at the International Trypanotolerance Centre (ITC) in The Gambia, West Africa.

2. REVIEW OF LITERATURE

2.1 Draught animals

2.1.1 The role of draught animal power in agriculture

In 1986 it was estimated that two billion people in developing countries were dependent upon draught animal power, either to cultivate their land, or for transport (Ramaswamy, 1986). In India two-thirds of the rural energy for cultivation is said to come from draught animals and in China animals are used in the preparation of over half of the total area cropped, 100 million hectares (Ramaswamy, 1986). In Asia as a whole nearly 85% of the draught power used in agriculture comes from cattle and buffalo (Dharsana and Campbell, 1993). By contrast in sub-Saharan Africa although the use of draught animal power is on the increase following its introduction some 70 years ago (Pannin and Ellis-Jones, 1994; Starkey, 1994), farmers still rely heavily on manual labour. In 1987, 89% of the region's agricultural power came from humans, with only 10% being supplied by animals (FAO, 1987). World-wide oxen are the most numerous draught animals on farms, followed by water buffalo, which are used primarily in S.E. Asia (Pearson, 1993).

Although the use of petroleum-based power in agriculture has increased rapidly across the developing world in recent years, particularly in S.E. Asian countries (Campbell, 1989) and on larger commercial farms in Africa and Latin America, there are many areas and tasks where draught animals are the only viable and appropriate alternative to manual labour. These include areas where land holdings are small or the terrain difficult, places where cultivation operations must be carried out in water-logged fields. In such locations the use of draught animal power

is expanding (Pearson, 1993). Some farmers hire tractors for heavy work such as ploughing, but use their own draught animals for lighter tasks such as secondary cultivation, sowing and weeding. Thus draught animal power and petroleum-based power should be seen as complimentary rather than competing technologies.

Tractors are expensive to purchase, fuel and maintain. Spare parts often have to be imported, requiring good communications and scarce foreign exchange, commodities which at present are seldom available to subsistence farmers in the developing countries. Draught animals require a much smaller capital investment than tractors, can be fed on locally available feedstuffs, will replace themselves naturally and at the end of their working lives generally retain a value as meat. The drawback is that they must be fed, watered and cared for even when they are not being worked. Pearson (1993) noted that in some areas there are shortages of draught animal power because increasing human population pressure has resulted in more and more land being turned over to arable crops at the expense of grazing land.

Draught animals are employed in a wide variety of tasks in agriculture and transport including pulling carts, cultivating land, threshing and grinding grains, pumping water and hauling timber. The harnessing and equipment required for such operations has been reviewed in detail by Starkey (1989). Although the majority of draught animals are employed by subsistence farmers and small-holders, they are also used in commercial plantation agriculture and forestry, working alongside mechanical tools and vehicles (Dijkman, 1993; Kerr, 1986; Solberg and Skaar, 1987). Indeed Liang and Sarmin (1993) have demonstrated that in the Malaysian oil-palm industry buffalo-drawn carts are more efficient than tractors, maximising labour productivity, whilst reducing the labour required per unit area.

The number of days draught animals are worked each year varies considerably on individual farms. In a survey of Indonesian farming systems using draught animal power, Petheram (1991) found that buffalo used for cultivating rice and other crops

are typically worked for 3 to 5 h/day, 10 to 30 days per crop, with some farmers growing as many three crops a year using irrigation. In addition some of these farmers rented out their buffalo for between 100 and 200 days *p.a.* for tillage work. Buffalo used for haulage in S.E.Asia are often worked for to 200 - 300 days *p.a.* (Petheram 1991; de Guzman and Petheram, 1993). In semi-arid areas of Africa where cropping cycles are less intensive, draught animals are worked for far fewer days. For example, in western Zambia cattle are used for cultivation work for an average of only 46 days *p.a.* with a further 33 days pulling carts or sledges (Barrs, 1988) and in Mali oxen are worked for as little as 40 days *p.a.* (Khibe and Bartholomew, 1993).

Hiring or lending out draught animals is common practice in many parts of the world, increasing the income of the owners either directly or through payment in kind. In a survey in eastern Nepal in 1989 Gatenby, Pearson and Limbu (1990) found that 72% of farmers had borrowed or hired draught oxen.

2.1.2 Factors affecting work output

Work is a function of the force required to move an object and the distance through which that object is moved. Power is the rate at which the work is done and is therefore a function of time. The tractive or draught force required to pull, or push an object over a surface is equal to the draught resistance of that object, according to Newton's third law of motion. The draught resistance of an object comprises a vertical component, namely the weight of the object, and a horizontal component related to the shape of the object and the nature of the material through or over which it is being pulled.

The amount of work an animal does in a fixed time is determined by the draught force that animal generates and the speed at which it works. Two teams of animals pulling ploughs with the same draught force may have very different power outputs if one team is working faster than the other, although if they plough equal

land areas they will achieve the same work output albeit that one team will complete the task in a shorter time than the other. Animals pulling implements requiring only small draught forces to move them, are able to walk much further and often walk much faster than similar animals pulling implements with high draught forces, although the work output at the end of the day may be similar. A well constructed animal drawn cart has very little draught resistance to movement, enabling it to be pulled great distances over smooth surfaces without the animals having to do too much work. Consequently in Indonesia, buffalo pull carts loaded with bricks up to 30 km in an 8 h working day (Petheram, 1991).

The daily work output of a draught animal is influenced by many different factors and has been shown to vary considerably between individual animals or teams worked under similar conditions (Pearson, Lawrence and Ghimire, 1989). Starkey, (1989) considered those factors under three basic headings, (a) implement, (b) environment, and (c) animal factors, all of which he suggested affected a fourth category (d) operational and human factors. In this context, an implement is any piece of machinery or equipment powered by an animal, including carts. Implement factors are the physical dimensions, weight and design of the implement being propelled by the animal. Environmental factors include aspects of the climate, the time of day when the animal is working and the terrain or soil over or through which the implement is moved. Animal factors are such things as species, breed, size, age, weight, body condition, health, training, experience, temperament and recent work schedule. Operational and human factors relate to implement maintenance and adjustment, how the animal is harnessed to the implement and how the driver works the animal. Factors in each of the four categories interact to determine the draught force required by the animal to move the implement, the distance the animal will walk, the frequency and duration of rests, the walking speed and the time required to prepare the animal for work each day, from which the work and power outputs per day are calculated.

The draught force required to pull an implement can alter markedly with changes in the ground over or through which that implement is being pulled, and in the case of a cultivation implement the angle at which it is pulled through the soil. In an experiment in Costa Rica over a period of a fortnight, Lawrence and Pearson (1985) recorded draught forces varying from 589 to 2160 N for the same plough pulled through the same field by a single team of oxen. This variation was probably caused by one or more of the following factors; differences in soil and surface vegetation within the field; changes in soil moisture content across the field and over the time interval; or operational and human factors such as the angle of attachment of the plough to the animals and the skill of the driver in steering the plough. Pearson *et al* (1989) found that changes in ground conditions had a marked effect on the work output of oxen ploughing terraces in Nepal, through their effect on draught force. This study also demonstrated the influence the driver can have on the draught force required to pull an implement. When two different ploughmen used the same team of animals and the same plough to cultivate similar strips of land under similar environmental conditions the mean draught force recorded over one hour varied by up to 33%. One ploughman preferred to work the animals much faster than the other, and in doing so he must have must have altered the working depth or direction of the plough thereby reducing the draught force needed to pull it. The skill of the operator in setting up and steering an implement can have a significant effect on the draught force required to pull it and consequently on the work and power output of the animals. Calculations of daily work rates should of course include time spent harnessing and unharnessing animals and adjusting equipment (Starkey, 1989).

Driver motivation is important in determining working speed. If the driver is not motivated then the animals are unlikely to work hard, conversely if the driver wants to finish a particular task in a day, he or she may be tempted to overwork the animals affecting their work performance the following day. This is an obvious

problem when animals are hired by the day, consequently in some areas animals are always rented out with a driver (personal observation).

Animals often have to contend with marked fluctuations in draught force particularly when ploughing or hauling carts over rough ground or ground covered in vegetation. In laboratory experiments Lawrence and Stibbards (1990) observed that such fluctuations reduced an animal's willingness to work, although they did not affect the efficiency with which the task was completed.

Once the draught force required to pull an implement increases beyond a certain point, the working speed of the animal or animals pulling it falls and the frequency of rests may increase, until the draught force becomes so great that the animals are unable to move the implement at all. Starkey (1989) suggested that this reduction in speed with increasing draught force is particularly noticeable in working cattle. The point beyond which an animal starts to slow down is often referred to as the maximum sustainable draught force of that animal. The maximum draught force an animal is capable of exerting is related to its body weight, in the field this is affected by the surface on which the animal is walking and the harness used to attach the animal to the load. Estimates of the sustainable draught forces for different species of draught animals vary, but are generally between 10 and 15% of body weight (CEEMAT/FAO, 1972; Goe and McDowell, 1980) with a 7.5% reduction for multiple hitching. Starkey (1989) however suggested that such figures are of little practical value because the effect upon the animal or animals depends on how the force is assessed and upon the working regime used.

The relationship between draught force, speed of working and animal liveweight means that there is a positive correlation between animal liveweight and draught power output (Starkey, 1989). Consequently heavier animals pulling the same loads at the same speeds as lighter animals are working at a lower percentage of their maximal power output.

Species, breed, size, strength, training, fitness, health, experience, temperament and the amount of work done over preceding days can all influence the speed of working and the distance an animal is willing to walk in a day. Different species and breeds of animal walk at different speeds, probably because of anatomical differences resulting in variations in stepping rate and stride length (Pearson, 1989a). Horses will pull light carts at speeds of 1.5 ms^{-1} without breaking into a trot, whereas buffalo and cattle seldom exceed 1 ms^{-1} (Pearson, 1994). In the hills of eastern Nepal, Pearson (1991) found that cross-bred Jersey cattle worked faster than the indigenous oxen. Even within a group of animals of the same breed and of similar size and weight some will walk faster than others. Animals are no different to humans, some are temperamentally less suited to work than others. This was clearly demonstrated by Pearson *et al* (1989) monitoring the work output of five teams of oxen ploughing. As an animal becomes fitter and stronger it will work faster and cover longer distances, with experienced animals tending to work more consistently than novices (Pearson, 1989a). In regions where work is very seasonal, it may take several weeks for draught animals to regain their fitness at the start of the working season, particularly if they are in poor condition having been under nourished during the dry season lay off (Fall, 1995).

The effects of ill-health on work performance can be sub-divided into the effects of physical injury and of pathogenic disease. There is little doubt that because of the work they perform draught animals are particularly vulnerable to physical injuries (Hoffman and Dalgliesh, 1985), such as sores, foot problems, muscle, joint and tendon damage (Wells, 1986), all of which will reduce output if they don't actually preclude an animal from working altogether. Prevention and if necessary prompt treatment of injuries is a prerequisite of good draught animal husbandry (Dharsana and Campbell, 1993). The types of injuries commonly suffered by working animals have been described in detail by FAO (1972) and by Williamson and Payne

(1978). The effects of pathogenic disease on work performance are more difficult to determine accurately and are discussed in detail in section 2.1.4 below.

Climate and more specifically ambient temperature and relative humidity are the other important environmental factors which can affect work output in addition to terrain. The muscular activity required for work generates heat as a by-product of the oxidation of chemical energy (Upadhyay, 1989). That heat together with any heat gained directly from solar radiation or from the digestion of food, must be dissipated if a working mammal is to maintain homeostasis. This is achieved through non-evaporative or sensible losses (radiation, conduction and convection) and by evaporation (sweating, panting and drooling). The relative importance of each method depends upon ambient temperature and animal species (Mount, 1979). Working animals in the tropics lose heat primarily through convection and evaporation, however as environmental temperatures increase convective losses decrease as they rely on a temperature difference between the animal and the environment. High relative humidities reduce evaporative losses which depend on water vapour differences (Mount, 1979). An animal that is unable to maintain a stable core body temperature through convective and evaporative losses will seek to modify its behaviour in an attempt to reduce its heat production and increase its heat loss. Working animals will slow down and try to stop work (Fall, 1995; Pearson, 1989a; Upadhyay and Rao, 1985). In addition elevated body core temperatures may actually accelerate the onset of fatigue by impairing muscle function (Nielsen, 1989). An animal starting work with an elevated body temperature is likely to become heat stressed and fatigued more rapidly than one starting work with a normal body temperature, with a consequent effect on work output.

In regions where there are large diurnal fluctuations in temperature and relative humidity, farmers generally avoid working their animals during the heat of the

day. In Indonesia hauliers using buffalo to pull carts over long distances actually prefer to work their animals through the night (Winugroho, 1991).

There are considerable breed and species differences in both heat tolerance and ability to lose heat. Buffalo are regarded as particularly heat intolerant, possessing fewer hair follicles and fewer sweat glands than cattle (Hafez, Badreldin and Shafei, 1955), giving them less protection from solar radiation and rendering them less able to loose heat through evaporative cooling (Moran, 1973). Consequently in high ambient temperatures draught buffalo become overheated and refuse to continue working much more quickly than cattle (Bakrie, Murray, Hogan and Kennedy, 1995; Pearson, 1989a) unless they are first allowed to wallow or are sprayed with water to enhance evaporative cooling. The time required for wallowing can reduce the work output per day of buffalo compared to cattle in hot climates (Pearson, 1989a). The critical body temperature above which a buffalo will refuse to work appears to vary between individuals (Pearson, 1989a). Amongst cattle physiological and anatomical studies by Macfarlane, (1968a and b) have shown that *Bos indicus* animals are better adapted to hot climates than *B. taurus* animals. Work by Thomas and Pearson (1986) and by Pearson (1991) has suggested that where draught animals are required to work long hours in high temperatures and relative humidities, indigenous cattle may have a higher work output than larger European crossbreds because of their superior heat tolerance. Recently Fall (1995) has demonstrated that body condition can also have a marked effect on the heat tolerance of working oxen, with fatter animals being less able to maintain homeostasis than their thinner contemporaries probably due to subcutaneous fat restricting dissipation of heat from the body core to the environment.

2.1.3 The physiology of work

Work involves the conversion of chemical energy from food or stored body energy reserves, into mechanical energy seen as muscular contractions. Within muscle tissues chemical energy is stored as adenose triphosphate (ATP). Muscular contractions are fuelled by the energy liberated from the reduction of ATP with water regulated by the enzyme myosin ATPase. This reaction also produces adenosine diphosphate (ADP), which must be phosphorylated to replenish the very limited supplies of ATP in skeletal muscle if contractions are to continue for more than a second or two (McMiken, 1983). As the speed of muscle contractions increases so the rate of ADP production increases. If an animal is not working hard sufficient oxygen can be supplied to muscle tissues via the bloodstream to maintain aerobic phosphorylation of ADP to ATP, however if it is working at or near its maximum output, the supply of oxygen to the tissues may be insufficient to meet the demands for aerobic phosphorylation. In this situation to avoid ATP shortages the process becomes predominantly anaerobic.

At the sub-maximal levels of exercise normally required of draught animals, aerobic metabolism predominates (Pearson, 1985) with anaerobic metabolism being used to supply short term increases in work rates, such as might be required to set a cart in motion or pull it over an obstacle (Pearson and Archibald, 1989). Prolonged anaerobic metabolism is not sustainable and leads rapidly to fatigue and reduced work output as supplies of substrates required to fuel the process become exhausted and lactic acid builds up in muscle tissues. It is therefore important to maximise aerobic metabolism and to work animals within their aerobic limits whenever possible, conserving anaerobic energy reserves. Maximum aerobic metabolism varies with body size, with genetic inheritance and with training, hence the variations in work performance seen between species, breeds and individuals (McMiken, 1983). Energy

metabolism and consequently work output, are enhanced by training and good nutrition and impaired by fatigue, malnutrition and disease.

The physiological limitations on aerobic metabolism in the skeletal muscles of mammals are determined by the supply of oxygen and metabolites to muscle tissue, the oxidative capacity of that tissue and the subsequent removal of waste products. The oxidative capacity of muscle tissue is a function of the oxidative enzymes and mitochondria in the muscle, both of which increase with training (Åstrand and Rodahl, 1977; Hoopeler, Luthi, Cluassen, Weibel and Howald, 1973). Oxygen supply is affected by respiration, cardiac output and the oxygen carrying capacity of the blood, all of which can be enhanced by training and increase with exercise although to varying degrees in different breeds and species of animal. Hence the concentration of red blood cells in an animal's circulatory system can have a marked effect on its' oxidative capacity and ability to maintain aerobic metabolism. Horses are able to increase their aerobic metabolism by up to 36 times in response to exercise (Thomas and Fregin, 1981), compared to only a two fold increase in man (Åstrand, 1976) and dogs (Wagner, Horvath and Dahms, 1977). The increase seen in the horse is achieved primarily by exercise induced contraction of the spleen (Persson and Bergsten, 1975) which dramatically increases circulating red blood cell numbers (erythrocytes), packed cell volumes and haemoglobin concentrations and hence the oxygen carrying capacity of the blood. In cattle increases in red blood cell numbers with exercise are small or non-existent (Hays, Bianca and Naf, 1978; Pearson and Archibald, 1989; Singh, Soni and Bhattacharyya, 1968), suggesting that exercise induced splenic contraction is not significant in bovines. Breed differences in respiration rates and cardiac output leading to differences in work output and the onset of fatigue have been demonstrated in cattle by Upadhyay and Madan (1985), who compared Haryana and Haryana × Holstein crossbred oxen.

In addition to its effect on red blood cells, exercise has also been demonstrated to cause changes in white cell numbers and function through its action as a stressor stimulating neuroendocrine responses, which in turn acts upon the LRS (Griffin, 1989). These changes and their effect on immune function are discussed further in section 2.2.

2.1.4 Disease in working animals

Draught animals are susceptible to all the diseases that afflict their non-working contemporaries, however as Hoffman and Dalgliesh, (1985) and Wells, (1986) observed little is known about the relationship between disease and work. A number of authors have speculated that disease and particularly sub-clinical disease may considerably reduce the work performance of draught animals on farms (Hoffman and Dalgliesh, 1985; Pearson, 1989b; Rukmana, 1979; Starkey, 1989). Conversely it has also been suggested that draught animals may actually be more susceptible to infection than other animals (Dharsana and Campbell, 1993; Munzinger, 1982; Starkey, 1981), because of the action of work as a stressor increasing corticosteroid production which in turn can depress immune responses to a disease challenge (Stephens, 1980; Tizard, 1992). The problem may be exacerbated by the stress of under-nutrition (Wells, 1986). Protein deficiencies can impair immunoglobulin production directly (Kelley, 1980; Tizard, 1992).

The effects of some diseases on work output are obvious since they limit function directly, for example diseases causing lameness such as foot and mouth which is endemic in Asia and parts of Africa and Latin America, or more serious diseases which can cause high mortality and reduce the numbers of animals available for work, such as rinderpest and African horse sickness (Wells, 1986). Other diseases such as ephemeral fever seen in cattle and buffalo throughout the tropics and sub-

tropics of Africa, Asia and Australia, can render animals incapable of working for short periods due to incapacity or fever. (Hoffman and Dalgliesh, 1985) suggested that the potential effect of chronic sub-clinical disease on work output may however be far more serious than that of overt disease. Their reasoning was that sub-clinical disease can cause weight loss and that liveweight is positively correlated with power output, however not all sub-clinical infections cause weight loss. Such infections may go undiagnosed for months, or even years, particularly in areas without adequate veterinary services, since an animal without obvious signs of disease is usually assumed to be healthy. If the effect on work performance is either small or gradual, that too may go undetected.

Diseases prevalent in draught animals in different parts of the world have been catalogued by various authors (Hoffman and Dalgliesh, 1985; Partoutomo *et al*, 1985; Ravindran, Massaquoi and Wiles, 1993; Wells, 1986). Others have described particular disease episodes in working animals (Centre for Tropical Veterinary Medicine, 1976; Löhr, Pohlpark, Srikitjakarn, Thaboran, Betterman and Staak, 1985; Wells, 1982). There is however little quantitative data available on reductions in work performance due to disease, either in individual animals, or in terms of lost agricultural productivity. Rukmana (1979) claimed that swamp buffalo infected with Trypanosoma evansi ploughed 29% less land per hour than uninfected buffalo, following experiments in Java, Indonesia, but gave few details of the work. Pearson, (1989b) reported an increasing difference, up to 33%, in the power produced by two similar teams of buffalo carting loads in eastern Nepal, with the only apparent difference between the two teams being that the weaker pair were anaemic, which the author speculated may have been due to a parasitic infection. Roberts *et al*, (unpublished) found that clinical fasciolosis reduced the work output of a group of buffalo by 7-15%.

In countries relying heavily on draught animals reductions in power output of 30% on a national scale, could cause serious economic losses through reduced crop yields, resulting in food shortages which might only be alleviated by expensive imports. Lun, Fang, Wang and Brun, (1993) reported that in China trypanosomosis in the draught animal population has in the past caused serious food shortages leading to malnutrition, although unfortunately no details were given. When attempting to assess the impact of a particular disease on draught animal power in a specific location it is however important to consider the whole farming system (Hoffman and Dalgliesh, 1985). A disease outbreak during the working season can be disastrous, particularly if the growing season is short and replacement animals are not available, whereas the same outbreak when the animals are not needed for work may only be of minor importance so long as the infected individuals recover.

Fasciolosis is acknowledged as a widespread problem in Nepal, which in the late 1970's was estimated to be costing the country between two and five million pounds sterling in lost livestock productivity *per se* (Morel and Mahato, 1987) with no allowance for losses incurred through the reduced work output of draught animals. In Indonesia in 1984, *T. evansi* was calculated to be costing the country US$ 28 million pa. in lost animal productivity including losses in draught power (Directorat Jenderal Peternakan, 1984). Following field studies in four provinces Samui and Hugh-Jones (1990) estimated that in 1985 dermatophilosis was costing Zambian farmers US$ 193 per draught ox infected, with 11% of the herds surveyed having at least one animal infected during the year. The estimated costs included losses due a reduction in the land area cultivated, loss of income from hiring out animals and the cost of treating the sick animal. Although 84% of the farmers questioned in this study actually kept spare oxen as insurance, they said that introducing a new animal into an existing team originally of four was not easy and that it often led to reductions in work output. The costs of keeping and feeding spare animals were not included in the

costs of the disease. Tsetse transmitted trypanosomosis still precludes or severely restricts the use of draught animals in large areas of sub-Saharan Africa (Chadega, 1994).

The suggestion that work may make draught animals more susceptible to disease is supported by epidemiological evidence from a number of countries. Starkey (1982) reported that in Sierra Leone trypanotolerant cattle which are normally highly resistant to streptothricosis may become susceptible to the disease when stressed by work. Similarly in a survey of the prevalence of *T. evansi* in buffalo in north-eastern Thailand Löhr *et al* (1985) found distinct peaks of acute infection shortly after the onset of the rains when animals were being worked hard and when biting fly numbers had risen dramatically. According to Wells (1986) following the Vietnam war there was a shortage of working buffalo so that animals that were available were worked exceptionally hard. This led to clinical episodes of trypanosomosis with significant mortality in the Red river delta where for several decades previously the disease had little economic impact. In a laboratory experiment Anderson, Youanes, Vestweber, King, Klemm and Kennedy (1991) showed that stressful exercise made Holstein yearling calves more susceptible to a subsequent pneumonic pasteurellosis challenge.

2.1.5 Assessment of work performance and energy expenditure

Work performance

Care must be exercised in designing experiments to measure work output because of the large number of factors which can affect the work performance of draught animals some of which were illustrated by Lawrence and Pearson (1993) in their review of experimental methods in draught animal research. There are two basic methods of assessing work performance in draught animals, one involves direct measurement of work done over time by electronic means, the other is a performance

type test with animals or teams pulling fixed loads, either over set distances with the time taken being recorded or conversely for set periods of time with distance travelled being measured. The first method is suitable for monitoring the work and power outputs of animals performing cultivation tasks in the field where draught forces may vary considerably depending upon the uniformity of the soil through which the implement is being pulled, as has been demonstrated by Pearson *et al* (1989), however it relies on complex and expensive electronics. The second method is particularly useful in experimental situations as it allows the performance of large numbers of animals to be compared simultaneously with little more than a tape measure and a stopwatch.

The field technique involves measuring the average draught force (ADF) required by an animal or animals to pull an implement and the distance over which it is pulled, from these two measurements the work done is calculated. To accomplish this Peter Lawrence based at the CTVM developed an electronic device known as an ergometer, comprising a load cell (Novatech Ltd. St. Leonards-on-Sea, UK) attached between the animals and the implement, to measure distance averaged draught force, and an odometer trailed behind the animals to measure distance travelled (Lawrence and Pearson, 1985). Data from these two sensors are fed to a data logger which integrates the draught force with the distance travelled to calculate the total work done. O'Neill, Hayton and Sims (1989) constructed the AFRC - Engineering draught animal performance data-logger to perform the same task. Draught force can be measured on a time or distance averaged basis, however Lawrence and Pearson, (1985) demonstrated that the time averaged method is unsuitable for measuring the work output of draught animals in the field where draught force may vary considerably. Using an ergometer the working speed and power output of a team of animals can be calculated from measurements of the work done and the time taken to complete the work. This technique gives an accurate measure of work output, but

only a single animal or team can be monitored at any one time unless several ergometers are available. To compare the work output of several teams of animals a series of measurements must be made either on the same day or on successive days with the possibility that changes in environmental conditions such as air temperature or soil moisture content may affect the results. Such changes were observed by Pearson *et al* (1989) in their experiments in Nepal.

In the performance type test individual animals or teams pull identical carts or sledges each loaded to a fixed percentage of animal liveweight around a prescribed circuit. Working the teams simultaneously under these conditions ensures that the ADF required by each team to pull its load is a similar percentage of animal liveweight. The time taken to complete a number of laps of the circuit or the distance travelled in a set time is recorded and compared between teams as a measure of the work performance. Speeds of working can also be compared. As draught power output is positively correlated to animal liveweight (Starkey, 1989) loading each sledge or cart to a fixed percentage of team liveweight ensures that all teams are worked at the same proportion of their potential maximum draught power output. Although it is less accurate than using an ergometer, this technique allows the work performance of larger numbers of animals to be compared simultaneously under identical conditions eliminating possible temporal effects as illustrated by Bartholomew, Khibe and Little (1994) in Mali and Komarudin-Ma'sum, Teleni, Martin and Affhandy (1995a and b) in Indonesia. Furthermore assuming that the track surface does not change thereby altering the ADF required to pull each sledge, the technique also allows large numbers of animals in different experimental groups to be worked at the same intensity over longer periods of time without temporal or spatial differences. Attaching an ergometer to one of the teams in this type of test allows any changes in ADF which do occur over the course of an experiment to be monitored. Pearson (1989a) used this performance test technique to compare the

performance of cattle and buffalo carting loads in hot conditions in Nepal, although in that experiment the animals were worked on separate days.

Energy expenditure

There are a number of possible methods for measuring the energy expenditure of working animals, these have been reviewed in some detail by Lawrence, Pearson and Dijkman (1991). The factorial method developed by Lawrence (1985) remains the most practical method of estimating the energy expenditure of large numbers of draught animals in the field, although three portable breath by breath gas analysis systems have recently been developed by Clar (1991), by Lawrence *et al* (1991) and by Howell and O'Neill (1990) to accurately measure the energy expenditure of individual animals in the field, using technology originally designed for humans.

2.2 Stress & immunity

2.2.1 Stressors & stress

For an animal to survive it must maintain homeostasis irrespective of changes in its external environment. An external stimulus that challenges homeostasis is termed a stressor, the consequent biological response of the animal is the stress response (Moberg, 1985). Stressors can be chemical including malnutrition and disease, physical including exercise or conversely restraint, and psychological normally fear. It was originally thought that all stressors caused a single host response involving stimulation of the pituitary - adrenal - cortical axis leading to the non-specific production of glucocorticoids (Selye, 1946). But work by Mason (1968) in monkeys subsequently demonstrated that different stressors produce characteristically different responses and that stress provokes an autonomic adrenomedullary response as well a pituitary adrenocortical response. It is now accepted that different chemical, physical and psychological stressors cause different endocrine responses, which vary between and even within animal species (Dantzer and Mormede, 1985), causing changes in behaviour, physiology and immunocompetence (Griffin, 1989).

This review focuses primarily on the effects of exercise and nutritional stressors on immune responses and susceptibility to disease. The effects of a wide range of other stressors on immune responses and consequent susceptibility to disease in domestic animals have been reviewed by Kelley (1980).

2.2.2 Immune responses to pathogens

Immunology and the immune responses of mammals is a vast and complex subject about which much has been written, only a very simple outline is given here. Further details can be found in the many textbooks and papers on the subject, for example Roitt (1980) and Tizard (1992).

The immune system of mammals consists of two basic lines of defence against invading organisms such as bacteria, parasites and viruses; a non-specific response in which an invading organism is merely recognised as foreign and destroyed either directly or following phagocytosis; and a specific response in which the foreign antigen is identified and specifically targeted. Non-specific responses involve macrophages, granulocytes and natural killer (NK) lymphocytes. Specific responses involve the trapping and presentation of the foreign antigen to T and B lymphocytes by macrophages and other antigen presenting cells. Once the lymphocytes recognise the antigen they multiply and differentiate, T lymphocytes into cytolytic T effector or regulator cells (T helper and T suppressor cells), B lymphocytes into antibody producing plasma cells. The invading organism is then neutralised and destroyed by specific humoral and cellular responses. Both arms of the immune response, specific and non-specific, are mediated by cytokines, polypetide hormones which act as signals between the cells of the immune system.

2.2.3 Stress and disease susceptibility

Stress may increase an animal's susceptibility to disease through its action on the lymphoreticular system (LRS), suppressing immune responses (Griffin, 1989; Kelley, 1980). Anderson *et al* (1991) demonstrated that short periods of stressful treadmill exercise made a group of Holstein cattle more susceptible to experimental pneumonic pasteurellosis by affecting neutrophil function. The effects of transport

stress on the same disease in cattle are also well documented (Hambdy, Trapp and Gale, 1963; Hoerlein, 1980), hence its alternative name 'shipping fever'. Monkeys strenuously exercised whilst in the incubation phase of poliomyelitis have been shown to develop a higher incidence of severe paralysis than unexercised controls (Levinson, Milzer and Lewin, 1945), similarly enforced swimming raised the mortality rate of mice infected with Coxsackie virus from 5 to 50% (Gatmaitan, Chason and Lerner, 1970). In humans exercise has been implicated in outbreaks of infectious hepatitis (Morse, Bryan and Murle, 1972), polio (McCormick, 1942; Weinstein, 1973) and aseptic meningitis (Baron, Hatch, Kleeman and MacCormack, 1982). Several studies have also indicated that regular strenuous exercise such as that undertaken by marathon runners, is related to an increased incidence of upper respiratory tract infections (Nieman, Johansen, Lee, Cermak and Arabatzis, 1988; Peters and Bateman, 1983).

Kelley (1980) and Keusch, Wilson and Waksal (1983) cited evidence of the effects of malnutrition on immune responses in a variety of animal species, however less appears to have been written about actual disease outbreaks. Overfeeding is reputed to increase the incidence of infection in lactating (Morrow, 1976), and fattening cattle (Jensen and Mackey, 1979), whereas underfeeding has been demonstrated to increase the susceptibility of mice to the dwarf tapeworm *Hymenolepis nana* (Weinmann and Rothman, 1967). Nathan, Heller and Perek (1977) reported that 48 h starvation reduced antibody responses to *Escherichia coli* in chickens, but only when the birds were immunised within 24 h of starvation. In 1974, Cooper, Good and Mariani suggested that famine and pestilence had long been associated in humans although they did not provide evidence to link the two. Reductions in cellular immunity in both children (Geefhuysen, Rosen, Katz, Ipp and Metz, 1971) and adults (Bistrian, Blackburn, Scrimshaw and Flatt, 1975) associated with protein and energy deficiencies were however recognised around that time.

More recently Chandra (1980) observed that infections such as herpes simplex, measles, malaria, tuberculosis and certain gram negative bacterial infections were common causes of morbidity and mortality in people suffering from kwashiorkor (acute protein deficiency).

Stressors need not always adversely affect biological functions. Golub and Gershwin (1985) noted that stress may enhance as well as depress immunocompetence. Moderate exercise has been shown to enhance antibody responses (Liu and Wang, 1987) and increase tumour resistance, slowing tumour growth in mice (Good and Fernandes, 1981). There is also a widely held belief that moderate exercise improves human health, by increasing disease resistance (Fitzgerald, 1988), although as McDowell, Hughes, Hughes, Housh and Johnson, (1992) pointed out the evidence for this is largely anecdotal. A three month programme of moderate exercise did improve laboratory measures of immune response in a group of elderly Canadians aged 65 - 100 and resulted in them having fewer days in hospital with respiratory tract infections than age-matched controls (Chandra, 1989).

Stress may also affect the efficacy of vaccinations, leading Matthewman, Dijkman and Zerbini (1993) to suggest that draught animals should not be vaccinated when work stress was likely to interfere with immune responses to the vaccine. Unfortunately the effects of stress on the efficacy of vaccination are not clear and there appear to be few reports of such studies in large ruminants. Vessey (1964) demonstrated that stressors such as noise, light, movement or housing conditions could suppress antibody production in mice following immunisation. Solomon (1969) observed a depression of primary and secondary antibody responses in acutely stressed rats, however chronic low-grade stress caused by low-voltage electric shock actually increased antibody production. Similarly cattle given glucocorticoids simultaneously with bovine viral diarrhoea vaccine appear to develop better

subsequent disease resistance (Roth and Kaeberle, 1983); glucocorticoids are normally released by the adrenal cortex in response to stress and are recognised immunomodulators (Golub and Gershwin, 1985; Griffin, 1989). Spalatin and Hanson (1974) found that 24 hours starvation enhanced antibody responses to Newcastle disease vaccination in chickens, although unfortunately it had no effect on the subsequent survival of the vaccinated birds when challenged.

2.2.4 Neuroendocrine responses to stress

Based on work by Kagan and Levi (1974), Moberg (1985) proposed that an individual's response to a stressor should be considered in three distinct stages each of which involves a series of biological processes:

1. Recognition of a threat to homeostasis by the central nervous system (CNS).

2. The stress response, including the organisation and activation of behavioural, autonomic and neuroendocrine responses.

3. The consequences of the stress response, namely a change in biological function which although intended to reduce or remove the original stressor and re-establish homeostasis, may also lead to a prepathological state and possibly to the development of pathology. Pathology is considered to include disease, impaired reproductive ability, reduced growth rate, loss of weight or abnormal behaviour deleterious to the animal, such as self mutilation.

Moberg (1985) suggested that the magnitude and duration of the stressor determines whether or not an individual's stress response proceeds to the prepathological stage. The biological cost to an animal of maintaining homeostasis in the face of a major or persistent stressor may be disruption to its mental or physical systems, manifested perhaps as aberrant behaviour, infertility or immunosuppression. The longer an animal remains in a prepathological state the more likely it is to develop

a pathology (Kagan and Levi, 1974); immunosuppressed animals may become infected; infertile animals may miss the opportunity to reproduce; and animals behaving abnormally may injure themselves or others. The effects of exercise in man illustrate the importance of the magnitude and duration of the stressor. Acute exhaustive exercise and chronic long term training such as is undertaken by professional athletes have both been shown to cause post-exercise immunodepression whereas moderate exercise may actually enhance immune responses (Fitzgerald, 1988; Lewicki, Tchórzewski, Denys, Kowalska and Golinska, 1987; Pedersen, 1991).

Recognition of a stressor by the host CNS triggers a graded response involving four distinct neuroendocrine systems (Griffin, 1989) all of which can ultimately affect the LRS and consequently immunocompetence. The four systems illustrated in Figure 2.1 are; I. autonomic nervous stimulation; II. hypothalamic-pituitary-adrenal axis responses; III. neuropeptide and neurotransmitter production; IV. neuroimmunological peptides and receptors. The latter permit bidirectional signals to be transmitted between the CNS and the LRS, thus substances produced by activated cells within the LRS in response to an immunological challenge may subsequently affect CNS function (Besedovsky and Del Rey, 1986; Carr and Blalock, 1986). The four systems described here are discussed in detail by Griffin (1989).

According to Hoffman-Goetz (1992) the stress of exercise produces a cascade of co-ordinated hormonal responses that affect the behaviour of immune cells. Initially activation of the autonomic nervous system results in the production and discharge into the plasma of the catecholamines, adrenaline and noradrenaline. Noradrenaline is primarily released from sympathetic nerve endings although small quantities are also released from the innervated adrenal medulla along with adrenaline. These catecholamines have pronounced physiological effects on cardiovascular function and metabolism and ultimately on blood flow through lymphoid tissues and on leukocyte circulation patterns (Hoffman-Goetz and Pedersen, 1994).

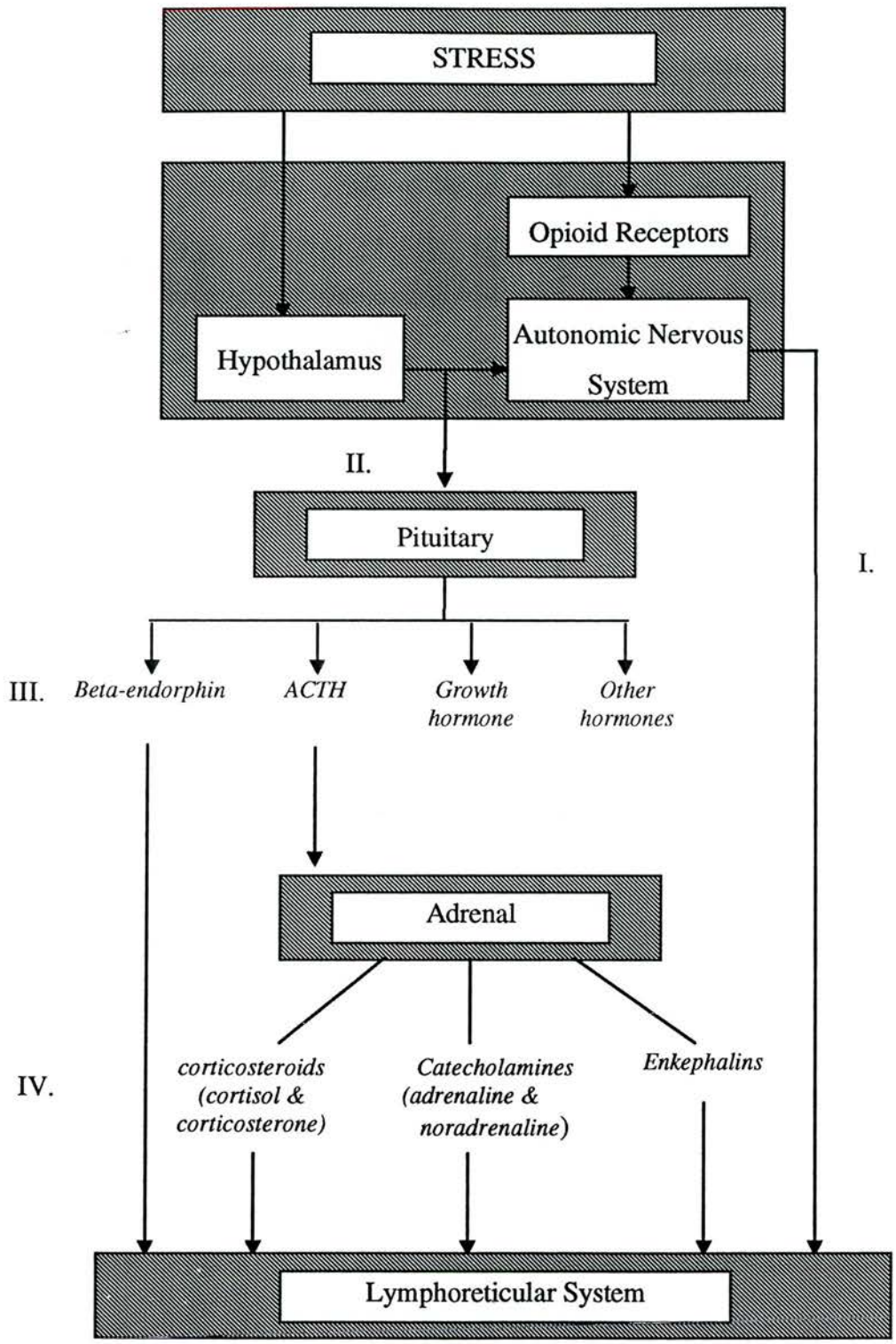


Figure 2.1 : Stress and immunomodulation (after Griffin, 1989)

The second stage of the response involving stimulation of the hypothalamic-pituitary-adrenal axis leads to the secretion of adrenocorticotrophic hormone (ACTH), which in turn stimulates the adrenal cortex to release glucocorticoids into the circulation. Glucocorticoids (cortisol and corticosterone) affect most of the homeostatic systems in the body, although they act primarily on gluconeogenesis, and on inflammatory and immune responses (Griffin, 1989). Leukocyte responses to corticosteroids vary between animal species depending upon the number of lymphocytes the animal possesses (Griffin, 1989). This is an important consideration when comparing the stress responses of different species. In animals with relatively high numbers of lymphocytes such as laboratory rodents and chickens the release of cortisol into the circulatory system results in a lymphopaenia and neutrophilia with a net decrease in leukocytes, whereas those with relatively few lymphocytes such cats, dogs, cattle pigs, horses and man respond with a leukocytosis caused by an increase in neutrophils (Blecha, Barry and Kelley, 1982; Gwazdauskas, Pape, Peery and McGillard, 1980).

Glucocorticoids are also recognised as potent immunosuppressors affecting cytokines, monocytes, macrophages and NK cells (Hoffman-Goetz, 1992). They have been shown to reduce humoral immunity (Gwazdauskas, Gross, Bibb and McGillard, 1978) and *in vitro* lymphocyte blastogenesis (Roth, Kaeberle and Hsu, 1982). Cattle injected with glucocorticoids become more susceptible to a wide range of diseases including coccidiosis (Niilo, 1970; Stockdale and Niilo, 1976), herpesvirus (Sheffey and Davies, 1972), fatal bovine diarrhoea (Shope, Muscoplat, Chen and Johnson, 1976) and *Babesia argentina* (Callow and Parker, 1969). The effects of exercise and training on glucocorticoids have been reviewed by Tharp (1975).

In addition to catecholamines and corticosteroids, stress triggers the release of a number of other neurohormones and neuropetides including the opioid peptides, endorphins and enkephalins (Haynes and Timms, 1987; Lewis, Shavit, Terman,

Nelson, Martin, Gale and Liebeskind, 1985). The actions of these neurohormones and neuropetides on host physiology have been reviewed by Breazile (1987), their immunomodulatory effects were summarised by Griffin (1989) and by Goetzl, Adelman and Spreedharan, (1990).

2.2.5 Stress & cell mediated immunity

Kelley (1980) postulated that stress can have a major impact on cell mediated immune responses because of the sensitivity of T-lymphocytes to most of the hormones and peptides released by the CNS in response to a stressor (Besedovsky and Del Rey, 1986; Carr and Blalock, 1986; Cupps and Fauci, 1982; Riley, 1981). As Tvede, Kappel, Klarlund, Duhn, Halkjoer-Kristensen, Kjoer, Galbo and Pedersen, (1994) observed physical exercise has been demonstrated to induce dramatic changes in the cellular arm of the immune system in a large number of studies in both man and animals. Exercise causes a marked but transient increase in circulating leukocytes (leukocytosis) attributable primarily to an increase in neutrophils (neutrophilia) and lymphocytes (lymphocytosis) (Hoffman-Goetz and Pedersen, 1994). It can also have pronounced effects on other leukocyte subpopulations and on lymphocyte function (Nieman, Berk, Simpson-Westerberg, Arabatzis, Youngberg, Tan, Lee and Eby, 1989; Oshida, Yamanouchi, Hayamizu and Sato, 1988), however results appear to vary depending upon the fitness of the subjects, and the intensity and duration of the exercise undertaken (Gabriel, Schwarz, Steffens and Kindermann, 1992; Lin and Chen, 1993). Shinkai, Shore, Shek and Shephard (1992) suggested that differences in the timing of the assessments and assays used have also contributed to the apparent inconsistencies in the results. All of which make interpretation of the effects of exercise on cellular immune responses difficult.

Garrey and Butler (1929) postulated that in man the magnitude of an exercise-induced leukocytosis is proportional to the intensity of the exercise performed.

Recent studies by Nieman, Miller, Henson, Warren, Gusewitch, Johnson and Davis (1994) support this theory. To quantify the intensity of exercise used as a stressor, many authors have expressed the oxygen uptake of the subject whilst exercising as a percentage of that individual's maximum aerobic oxygen consumption (VO_2max). Thus (60% VO_2max, 1 h) indicates that the subject exercised for one hour at 60% of his or her maximum oxygen uptake. Exercise at less than 40% VO_2max is generally considered as light, 40 - 60% as moderate and 70 - 80% or above as severe (Nieman *et al*, 1994; Pedersen, 1991). Unfortunately however these divisions are not universally agreed and there is considerable overlap.

Both short exhaustive exercise lasting no more than 12 minutes (Wong, Smith, Thong, Opdebeeck and Thornton, 1992) and prolonged sub-maximal exercise, 80 km rides over 4 - 5 h, (Snow, Kerr, Nimmo and Abbot, 1982) have been shown to cause a leukocytosis in horses, associated with an increase in neutrophils, however there were marked differences in the rate of decline of neutrophils after exercise. Neutrophil counts continued to rise for 6 h after the short exhaustive exercise, before declining to pre-exercise levels by 24 h post-exercise, whereas they fell immediately after the long ride but were still above pre-ride levels 48 h later. The two exercise regimes also had different effects on lymphocyte numbers, but with both, changes were small and remained within the normal range for horses (Schalm, Jain and Carroll, 1975). Studies by Pearson and Archibald (1989) and Singh *et al* (1968), demonstrated that short periods (1-2 h) of sub-maximal exercise caused transient leukocytosis in cattle, although the proportions of the different leukocyte subpopulations did not change significantly in either study.

In man exercise leads to an absolute neutrophilia and lymphocytosis (Garrey and Bryan, 1935; Shinkai *et al*, 1992), often accompanied by a marked fall in eosinophils (eosinopaenia) (Nieman *et al*, 1989a; Nieman *et al*, 1994). Neutrophils can continue to increase for several hours post-exercise before falling back to pre-

exercise concentrations, whereas lymphocytes decline shortly after exercise ceases (Hoffman-Goetz and Pedersen, 1994). Two hours after strenuous bicycle exercise (75% VO_2max, 1 h) Tvede *et al* (1994) reported a fourfold increase in neutrophils and a 10% reduction in lymphocytes compared to pre-exercise values, in eight healthy untrained men. Eosinophil numbers are slow to recover after exercise. Nieman *et al* (1989a) found that 21 h after exhaustive exercise the eosinophil counts of experienced long distance runners were still only 60% of their pre-exercise values.

McCarthy and Dale (1988) suggested that circulating lymphocyte responses to exercise in humans are best described as biphasic, characterised by a lymphocytosis during and for a short time after exercise, followed by a lymphopaenia which can continue for several hours. Reviewing results from a number of other studies Nieman *et al* (1994) stated that vigorous exercise (70 - 80% VO_2max for 5 - 60 mins) leads to a 70 - 130% increase in lymphocytes immediately post-exercise, followed by a 30 - 50% fall in lymphocytes for up to 4 h thereafter. They went on to conclude that the intensity of the exercise determines the magnitude of lymphocytosis and subsequent lymphopaenia. A similar pattern of lymphocytosis followed by lymphopaenia is seen with prolonged (in excess of two hours) moderate exercise (Nieman *et al* 1989a).

Little information is available on the effects of exercise on basophil numbers. Cupps and Fauci (1982) reported they decrease slightly with exercise, however Nieman *et al* (1994) found no changes with either moderate or high exercise. According to Pedersen (1991) monocyte concentrations do not change during exercise, although they increase two or threefold after severe exercise. Studies by Nieman *et al*, (1989a) and by Tvede *et al* (1994) support this statement, however a more recent experiment by Nieman *et al* (1994) and work by Shinkai *et al* (1992) suggests that monocytosis can occur both during and after, moderate and severe exercise.

In addition to its overall effect on total numbers of lymphocytes, exercise also has differential effects on lymphocyte subpopulations (Hoffman-Goetz and Pedersen, 1994; Pedersen, 1991). As Tvede *et al* (1994) observed there have been many studies on the effects of exercise on human lymphocyte subpopulations, however the biphasic nature of the lymphocyte response coupled with differences in the timing of blood sampling and differences in exercise intensity make it difficult to discern response patterns. T lymphocytes numbers appear to increase slightly during or immediately after exercise (Gabriel *et al*, 1992; Lewicki, Tchórzewski, Majewska, Nowak and Baj, 1988; Nieman *et al*, 1994; Shinkai *et al* 1992; Tomasi, Trudeau, Czerwinski and Erridge, 1982) but because of changes in other subpopulations, the percentage of T cells in the total lymphocyte population decreases (Gabriel *et al*, 1992; Oshida *et al*, 1988; Tomasi *et al*, 1982). B lymphocytes responsible for humoral immune responses have been reported to increase fourfold after short maximal exercise (10 - 15 mins) (Hedfors, Holm and Ohnell, 1976; Steel, Evans and Smith, 1974). Smaller increases are seen after longer sub-maximal exercise (Gabriel *et al*, 1992; Nieman *et al*, 1989a). After exercise during recovery, B and T cell numbers fall below pre-exercise values (Nieman *et al*, 1994; Shinkai *et al*, 1992) and may not return to normal for 6 hours or more (Nieman *et al*, 1989a). Natural killer cells (NK cells) the third major type of lymphocyte and the first line of defence against viruses (Pedersen, 1985), bacteria and tumours (Tizard, 1992), are known to increase markedly during both moderate and severe exercise (Lewicki *et al*, 1988; Nieman *et al*, 1994; Pedersen, 1991). Pedersen (1991) suggested that NK numbers return to pre-exercise levels within 1 - 2 h post-exercise, however work by Nieman *et al*, (1994) demonstrated that they may actually be depressed for 3.5 h or more after both moderate and severe exercise.

A number of studies of mitogen induced *in vitro* blastogenesis have suggested that exercise may also affect the proliferative responses of T, B and NK lymphocytes (Nieman *et al*, 1989a; Nieman *et al*, 1994; Oshida *et al*, 1988; Tomasi *et al*, 1982;

Tvede *et al* 1994). Work by Shinkai *et al* (1992) appears to demonstrate that exercise does not however change the *in vitro* responsiveness of lymphocytes on a per cell basis. These alterations in response appear to be due instead to exercise induced changes in cell numbers. A theory supported by Pedersen (1991) and by Hoffman-Goetz and Pedersen (1994) in their reviews of the literature. Recently however Nieman *et al*, (1994) have demonstrated that high (80% VO_2max, 45 mins) but not moderate intensity exercise (50% VO_2max, 45 mins) does cause a depression in T lymphocyte function in conditioned runners one hour after exercise, which cannot be explained by numerical changes in lymphocyte subpopulations. The authors commented that this warrants further research to determine whether it is clinically significant. Exercise-induced reductions in T cell and NK cell responses would render individuals less able to recognise and respond to pathogens and in particular viruses (Fitzgerald, 1991).

Although blood neutrophil numbers increase with exercise, there is evidence that the bactericidal and phagocytic activity of both blood and alveolar neutrophils are impaired by exercise through the action of corticosteroids. Exogenous corticosteroids are known to reduce neutrophil chemotaxis (Peyser, Sanda, Chinea and Oronsky, 1974) and bactericidal activity (Dale, Fauci and Wolff, 1974). Transient reductions in neutrophil bactericidal and phagocytic activity lasting up to one week, have been seen in horses (Wong *et al*, 1992), cattle (Anderson *et al*, 1991) and man (Lewicki *et al*, 1987; Müns, 1994) following short bouts of maximal or exhaustive exercise. Lewicki *et al* (1987) demonstrated that at rest the neutrophil bactericidal activity of trained human athletes was significantly lower than that of untrained controls although there were no significant differences in neutrophil phagocytic activity. This led them to conclude that whilst neutrophil phagocytic activity appeared to be unaffected by repeated long term exercise, such exercise might inhibit or exhaust the mechanisms responsible for intracellular killing of bacteria, rendering sportsmen and women more

susceptible to such infections. Wong, Thompson, Thong and Thornton, (1990) demonstrated that the antimicrobial function of equine alveolar macrophages is also reduced by strenuous activity, affecting their role in pulmonary defence.

Kelley (1980) observed that studies of the effects of nutrition on the immune responses of animals have largely been confined to the influence of particular dietary components such as protein, with little work done on the effects of undernutrition *per se*. Furthermore most of the studies have focused on laboratory animals with little information available on nutrition and immunity in cattle (Fiske and Adams, 1985) or any other ruminants. The effects of malnutrition on human humoral and cellular immune responses have been studied in detail (Chandra, 1980; Stiem, 1980), however as Fiske and Adams (1985) commented the direct extrapolation to cattle (and other ruminants) of results obtained in humans and laboratory animals is not advisable because of the unique nature of ruminant nutrition.

In animals in general, severe undernutrition impairs cell mediated immune responses by inducing thymic atropy and reducing the secretion of thymic hormones (Tizard, 1992). In cattle severe undernutrition, resulting in a weight loss of 0.5 kg/d has been demonstrated to cause a 30% fall in circulating lymphocytes over 140 days, however it had no significant effect on *in vitro* lymphocyte blastogenesis (Fiske and Adams, 1985). In the same experiment dermal hypersensitivity responses to tuberculin were slightly but not significantly reduced in the undernourished animals compared to adequately fed controls, following sensitisation with Bacillus Calmette-Guerin (BCG). This response is regarded as classic indicator of the functionality of cell mediated immunity (Tizard, 1992). Work by Pollock, Rowan, Dixon, Carter, Spiller and Warenius (1993) has indicated that undernutrition might actually increase skin sensitivity responses in young calves immediately post-weaning, whilst depressing lymphocyte blastogenesis. These results must be treated with caution however because of the young age of the animals and because the low plane of

nutrition employed was not particularly low being adequate for at least maintenance. From their results Fiske and Adams (1985) concluded that undernutrition to the extent employed in their study neither consistently depressed nor enhanced cell mediated immunity in cattle.

2.2.6 Stress & humoral immunity

Having reviewed studies involving a wide variety of stressors Griffin (1989) concluded that acute stress tends to cause a transient reduction in antibody production, whereas chronic stress involving repeated exposure over a protracted period of time leads to an adaptive response which may actually enhance antibody levels. Kelley (1980) however suggested that the effects of stress on antibody synthesis are equivocal, citing heat stress in poultry as an example. The literature reporting serum immunoglobulin changes in humans in response to acute exercise is not clear cut, possibly due to the small numbers of subjects used in some studies and to failure to adjust for changes in plasma volume that occurred during exercise (Nieman, Tan, Lee and Berk, 1989; Stephenson, Kolka and Wilkerson, 1985). Most of the studies on the effects of exercise on immune responses appear to have been done in humans, particularly sportsmen and women, although some work has been done in horses used for sport and in laboratory rodents.

Both acute maximal and sub-maximal exercise have been reported to increase non-specific serum immunoglobulin titres in man immediately after exercise (Hanson and Flaherty, 1981; Mackinnon, Chick, van Es and Tomasi, 1988; Nieman *et al*, 1989b; Poortmans, 1970; Poortmans and Haralambie, 1979; Stephenson *et al*, 1985), although in a number of these studies the increases reported were not actually significant. Stephenson *et al* (1985) reviewing their own results and those from other studies concluded that some of the significant increases in immunoglobulins reported post-exercise were due to reductions in plasma volume caused by dehydration and

were not stress related responses. A view subsequently supported by Nieman *et al* (1989b). Increases in IgG and IgA reported by Poortmans (1970) following maximal cycle exercise and the increase in IgG reported by Poortmans and Haralambie (1979) after a long distance run could not however be attributed merely to changes in plasma volume. Stephenson *et al* (1985) suggested that the apparent discrepancy between the results obtained by Poortmans (1970) and by Poortmans and Haralambie, (1979) and those from their own work was due to differences in the methods used to calculate plasma volumes. This led Simon (1984) and MacKinnon and Tomasi (1986) to conclude that acute exercise appeared to have little effect on non-specific serum immunoglobulins in man. Similarly Wong *et al* (1992) have demonstrated that acute maximal exercise has no effect on the serum IgG, IgM and IgA concentrations of unconditioned horses.

In vitro studies with a range of bacterial and viral pathogens led Mackinnon *et al* (1988) to conclude that acute exercise does not affect antibody responses to specific pathogens in humans.

Fitzgerald (1988) has suggested that chronic exercise at high intensity such as might be undertaken by professional athletes during a long competitive season can lead to reduced serum IgG concentrations at the end of the season. Contradicting this theory Nieman *et al* (1989b) found no significant differences in the resting serum IgA, IgM and IgG concentrations of a group of experienced marathon runners who had run an average of 68 km/week for the previous 3 years and a group of sedentary controls, implying that long-term exercise does not affect serum immunoglobulins. Chronic exercise has been demonstrated to increase antibody responses to a specific pathogen in laboratory mice (Liu and Wang, 1987), although as Fitzgerald (1988) commented such animals do not provide a good model for the effects of exercise on immune responses since they are very under-exercised compared to their wild cousins. Fitzgerald went on to suggest an alternative interpretation of Liu and Wang's results, namely that a lack of exercise may have actually depressed antibody responses in the

control group compared to the exercised animals. Griffin (1989) stated that caution is required in extrapolating stress responses observed in laboratory rodents which are recognised as corticosteroid-sensitive (Parillo and Fauci, 1979), to primates and domestic animals such as cattle which are regarded as corticosteroid resistant (Roth and Kaeberle, 1982).

Salivary immunoglobulins are the first line of defence against upper respiratory tract infections (Tomasi, 1969; Waldeman, Small and Rowe, 1969). Prolonged or chronic exercise at high intensity has been shown to depress human salivary IgA concentrations (Tharp and Barnes, 1990; Tomasi *et al*, 1982). Studies by Mackinnon *et al* (1988) and McDowell *et al* (1992) have however suggested that the effect may be cumulative, with an exercise threshold below which IgA secretion is unaffected. Short-term acute exercise has been demonstrated to have both positive and negative effects on salivary immunoglobulins. This variation in response depending upon exercise intensity and duration led Tharp (1991) to speculate that the effects of exercise on salivary immunoglobulins might be affected by the metabolic energy source for the exercise (aerobic or anaerobic). Tomasi *et al*, (1982) and Mackinnon *et al* (1988) suggested that exercise induced suppression of salivary immunoglobulins explains the high frequency of upper respiratory tract infections reported by competitive athletes following periods of over-training or intense competition. Mackinnon, Ginn and Seymour (1991) have since demonstrated an actual link between exercise induced suppression of salivary IgA and episodes of upper respiratory tract infections in sportsmen.

According to Tizard (1992) undernutrition generally has little effect on humoral immunity. Severe starvation has however been shown to reduce serum antibody responses in both cattle (Fiske and Adams, 1985; Griebel, Schoonderwoerd and Babiuk, 1987) and man (Law, Dudrick and Abdou, 1973). Griebel *et al* (1987) reported reduced antibody responses in weaned calves fed only 50% of their maintenance energy requirements. Fiske and Adams (1985) observed a reduced

antibody response to chicken erythrocytes in year old steers losing 0.5 kg of body weight per day compared to control animals fed for a small liveweight gain. Antibody responses to *B. abortus* in the same animals were unaffected by malnutrition. Cooper *et al* (1974) observed the same differential response to these two antigens in malnourished mice. Fiske and Adams (1985) explained this difference by referring to the fact that in contrast to responses to foreign erythrocytes, responses to *B. abortus* are not dependent on thymus derived lymphocytes. Considerable thymic atropy, a common feature of malnutrition in many animals (Tizard, 1992), was observed in the underfed steers (Fiske and Adams, 1985). In contrast undernutrition had no significant effect on non-specific serum IgG or IgM in the cattle, nor did it affect the percentage of circulating lymphocytes bearing surface immunoglobulins. These findings coupled with data on cell-mediated immunity from the same experiment (detailed in section 2.2.5) led Fiske and Adams to conclude that undernutrition of the severity used in their study does not have a consistent positive or negative effect on either humoral or cell-mediated immunity in cattle. They suggested that malnourished cattle may not suffer from the profound suppression of cell-mediated responses and consequent increased disease susceptibility associated with protein energy deficiency in other species, because of ruminal digestion which by its action tends to prevent protein deficiency.

2.2.7 Factors influencing stress responses

A large number of factors can affect responses to a particular stressor, some of which may be particularly pertinent to immune responses and working animals. These factors can be considered in two general categories, host factors and external factors.

Host factors which affect responses to stress include genetics, sex, age, rearing, conditioning, perception and psychological factors. Evolution and breeding

have led to species and breed differences in stress responses (Griffin, 1989), hence the danger of extrapolating data between species noted by Fiske and Adams (1985). Within a species responses to the same stressor can vary considerably between individuals (Moberg, 1985) and furthermore they can be modified by selective breeding as has been demonstrated in pigs (Edfors-Lilja, Lundstrom, Nyberg and Rundgren, 1987). In an attempt to minimise variations between individuals some researchers have used siblings in their studies (Cooper *et al*, 1974; Fiske, and Adams, 1985). Males and females may respond differently to the same stressor (Joasoo and McKenzie, 1976; Schouten, Verschuur and Kemper, 1988). Stress responses are also affected by age; young calves are more susceptible to disease than older animals following a variety of stressors, including transport (Staples and Haugse, 1974) and coat wetting (Jennings and Glover, 1952). Experience and conditioning are particularly important as they affect an animal's perception of the threat posed by a stressor which Griffin (1989) declared to be the most important factor influencing the stress response. An unfamiliar stressor will evoke a very different response to that elicited by a routine stressor to which an animal has been habituated by training, as illustrated by Johnson and Moberg (1980) and Pearson and Mellor (1976). The importance of perception was clearly demonstrated by Seligman (1975) investigating behavioural responses of dogs to a noxious stimulus. Dogs that had previously been prevented from escaping the stimulus, ceased to try even when an easy escape route was subsequently offered. An animal's perception of stress and its subsequent responses to the stressor can also be influenced by bonding during rearing as Moberg (1985) illustrated in an experiment with two groups of monkeys reared with different mother substitutes. As well as modifying an animal's perception of a stressor, there is evidence that training can directly influence CNS responses to stress. In an experiment with young lambs Siegel and Moberg (1980) found that training reduced adrenocortical responses to electric shock but did not affect the behavioural flight response. Griffin (1989) suggested "because stress results largely from the

individual's perception of the threat posed by the stimulus rather than its nature *per se*, a cognitive or psychological component is central to all stress". The psychological component of stress has been most clearly illustrated in humans, although it applies to other animals. As early as 1956 Hill, Goetz, Fox, Murawski, Krakauer, Reifenstein, Gray, Reddy, Hedberg, St. Marc and Thorn (1956) found that human athletes responded differently to the same amount of physical exercise on race days compared to training days. Good draught animal trainers and drivers subconsciously seek to minimise psychological stress in their handling of the animals during rearing, training and working (personal observation).

External factors which can affect stress responses include the nature of the stressor itself, its intensity and duration, its timing relative to any other stressor (in this case an immunological challenge) and the possible summation of multiple stressors. The diverse effects of eight major stressors on immune function in animals have been extensively reviewed by Kelley (1980). The effects of exercise intensity and duration on immunological responses have been detailed in the two previous sections of this review (sections 2.2.5 and 2.2.6). Riley (1981) experimenting in mice demonstrated that the timing of a stressor relative to an immune challenge can have a profound effect on subsequent immune responses. Stress imposed before mice were inoculated with tumours was found to enhance immune responses inhibiting subsequent tumour growth, whereas stress after inoculation inhibited immune responses allowing the tumours to grow rapidly with lethal consequences. The idea that stressors can summate was proposed by Moberg (1976) who suggested that two or more stressors occurring together might be sufficient to cause prepathological changes in an animal conducive to the development of disease, where individually those same stressors would not impose a significant biological cost on the animal. In draught animals the stress of work combined with moderate undernutrition might be sufficient to lower disease resistance.

Finally, in monitoring immune responses to stress it should be remembered that the stress of blood sampling and other experimental procedures *per se* can cause marked changes in haematological and immunological parameters if they are not accomplished rapidly and with the minimum of manual restraint, as has been observed in a number of studies (Archer, 1974; Riley, 1981; Sakurai, Senta and Amada, 1967; Stewart, Clarkson and Stel, 1970). The stress response caused by taking blood can however be reduced by training (Pearson and Mellor, 1976).

2.2.8 Techniques for measuring immunological responses

There are many techniques for monitoring humoral and cellular immunity, some counting cell numbers and changes in the relative proportions of cells, some testing cell function and others assaying the products of immune responses. Listed below are some of the techniques that have been used to monitor the effects of exercise and nutrition on immunity, together with brief comments on their use in the field.

Cell mediated responses

There are two commonly used methods for monitoring changes in leukocyte numbers in response to exercise, differential cell counts of stained blood smears and direct or indirect immunofluorescence using fluorescein-conjugated monoclonal antibodies. Differential cell counts from stained smears require few facilities beyond a skilled eye and a good microscope, although the technique is laborious and time consuming as was implied by Pearson and Archibald (1989) monitoring haematological changes associated with work in oxen. It is not possible to differentiate between lymphocyte subpopulations using this technique. Direct and indirect immunofluorescence can be used to quantify subpopulations depending on the

availability of suitable monoclonal antibodies specific for the cells to be counted. This latter technique has been widely used to monitor leucocyte population changes in response to exercise in humans, for example by Gabriel *et al*, (1992), by Lewicki *et al*, (1988) and by Pedersen (1991). Cell populations are counted either manually using a phase contrast microscope, or automatically using a flow cytometer. The cytometer although expensive has the advantage of speed. Fluorescence activated cell sorting (FACS) which has evolved from flow cytometry enables individual cell subpopulations to be separated out for further screening on the basis of their surface fluorescence or size. The principles behind flow cytometry and FACS analysis have been outlined by Tizard (1992).

Lymphocyte functionality is usually assessed *in vitro* by examining responses to plant cell lectins (Pedersen, 1991) which act as mitogens stimulating blastogenesis. Different mitogens are used to stimulate T and B lymphocytes (Tizard, 1992). Nieman *et al* (1989a) measured spontaneous blastogenesis in whole blood samples immediately after exercise using radioactive markers as an indication of *in vivo* stimulation of lymphocytes. Although both techniques have been widely used in studies of exercise and immunity Nieman *et al* (1994) commented that it is often difficult to compare the results of lymphocyte proliferation tests performed by different laboratories because of differences in the assay methods used, a view supported by Kelley, Osborne, Everman, Parish and Hinrichs (1981).

A variety of techniques have been used to characterise the effects of exercise on neutrophil function. Lewicki *et al* (1987) and Rodriguez, Barriga and De la Fuente (1991) examined the effects of exercise on neutrophil adherence, an important antimicrobial defence mechanism, using the methodology developed by MacGregor, Spagnudo and Lentnek (1974). Wong *et al* (1992) looked at chemotaxis and chemiluminescence, the former being one of the initial methods by which neutrophils are attracted to the site of an infection, the latter being an indication of their oxygen-

dependent killing capacity. Lewicki *et al*, (1987) and Roth *et al* (1982) used radioactively labelled *Staphylococcus aureus* to determine neutrophil phagocytic activity. Phagocytosis can also be measured using a methylene blue staining technique as Rodriguez *et al* (1991) demonstrated looking at phagocytic activity against *Candida albicans*.

Tizard (1992) suggested that the simplest method of testing cell mediated immune responses in the field is by intradermal skin testing since it can be performed with the minimum of equipment and without recourse to a sophisticated laboratory. Griffin (1989) stated that such tests remain the only practical way of monitoring cell mediated responses *in vivo*, although he warned that it is often difficult to obtain accurate quantitative results. Skin tests have been extensively used to examine the effects of exercise and nutrition on cell mediated responses for example by Bistrian *et al* (1975), Fiske and Adams (1985), Kelley, Randall, Greenfield, Evermann, Parish and Perryman, (1982), Pollock *et al* (1993).

Humoral responses

Although there are a variety of techniques for detecting and measuring antibodies, only two appear to be commonly used for assaying the effects of exercise on antibody responses, radial immunodiffusion and enzyme linked immunosorbent assays (ELISA). Laser nephelometry has been used by Nehlsen-Cannarella, Niemen, Jessen, Chang, Gusewitch, Blix and Ashley (1991) and by Nieman *et al* (1989b) to monitor serum antibody levels in humans.

Radial immunodiffusion assays rely on measuring the ring of precipitation which forms when antigen is allowed to diffuse into agar impregnated with a specific antiserum (Mancini, Carbonara and Heremans, 1965). The area of the ring is directly proportional to the amount of antigen placed in the well. By using known amounts of antigen a standard curve can be constructed, against which the areas of diffusion of

unknown samples are compared. The accuracy of the technique relies upon obtaining well defined, measurable rings of diffusion. The technique has been used to measure IgG, IgA and IgM in human serum and saliva before and after exercise (Schouten *et al* 1988; Stephenson *et al*, 1985) and in horse serum (Wong *et al*, 1992).

Nowadays ELISA is the most commonly used method of assaying antibodies. The technology has been extensively documented by many authors including Kemeny (1991 and 1992), Kemeny and Challacombe (1988), Voller, Bidwell and Bartlett (1979). Antibody ELISAs involve quantifying the reaction of serum, saliva or other antibody containing fluid, with an antigen which has been adsorbed onto polystyrene plates. To measure the binding which occurs the resulting antigen-antibody complex is incubated with an enzyme-coated conjugated antiglobulin to the species of animal from which the serum or saliva was collected. This antiglobulin in turn binds to the antibodies attached to the antigen. By using class-specific conjugated antiglobulins the different classes and sub-classes of immunoglobulin can be measured. Finally the amount of antiglobulin bound to the serum is visualised by the addition of an enzyme substrate and chromogen, the resultant colour change being quantified using a spectrophotometer to measure the absorbance of light or optical density (OD). An additional layer can be added to this assay if an appropriate class specific conjugated antiglobulin is not available or alternatively to increase assay sensitivity. Kemeny (1992) suggested that ELISAs offer many advantages over other immunoassays in that they are fast, sensitive and convenient, furthermore the reagents are cheap to prepare and highly stable (Voller et al 1979), however as Wright (1987) observed quality control is crucial to accuracy.

2.3 Trypanosomosis

2.3.1 Introduction

Trypanosomosis or trypanosomiasis is the general name for a group of debilitating and sometimes fatal, parasitic diseases caused by Protozoa of the genus *Trypanosoma*, order *Kinetoplastida* which afflict a wide variety of mammals including man in many regions of the world (Stephen, 1986). The diseases caused by trypanosomes are infectious, virulent and inoculable, but with one exception (dourine a venereal disease of equines) they are not contagious (Itard, 1981). Trypanosomes are obligatory extracellular parasites which multiply in the blood, lymphatic vessels and tissues of their hosts causing a chronic wasting disease typified by fluctuating parasitaemia, profound anaemia and general loss of condition (Molyneux and Ashford, 1983). Secondary effects of infection can include reduced growth rates (Fox, Mmbando, Fox and Wilson, 1993), a decline in milk production (Agyemang, Dwinger, Jeanin, Leperre, Grieve, Bah and Little, 1990), decreased fertility and reproductive problems (Löhr, Pohlpark, Siriwan, Leesirikul, Srikitjkarn and Staak, 1986; Stephen, 1966), a reduced capacity for animal traction (Bourn and Scott, 1978; Rukmana, 1979) and increased susceptibility to other diseases (Fox *et al* 1993).

All mammalian trypanosomes except *Trypanosoma equiperdum* which causes dourine, are transmitted from one host to another via hematophagous insect vectors (Itard, 1981), although in Latin America the vampire bat has also been implicated in the transmission of one species of trypanosome (Hoare, 1965). Depending upon the species of trypanosome, transmission can be either mechanical without the parasite developing in the fly vector, or cyclical following an obligatory cycle of development within the fly (Table 2.1).

Species	Definitive host	Vector	Mode of transmission	Distribution
T. vivax	cattle, sheep, equines, goats, antelopes, dogs	tsetse flies (outside Africa. other hematophagous flies)	cyclical, (mechanical outside Africa)	Tropical Africa, also Mauritius, Antilles and S. America
T. uniforme	antelopes, cattle, sheep, goats,	tsetse flies	cyclical	Central & E.Africa, Angola
T. congolense	cattle, sheep, zebras, warthogs, equines	tsetse flies	cyclical	Tropical Africa
T. simiae	warthogs, pigs, camels; possibly cattle, equines	tsetse flies	cyclical	Tropical Africa
T .suis	pigs	tsetse flies	cyclical	Zaire, Tanzania
T. b. brucei *	domestic mammals	tsetse flies	cyclical	Tropical Africa
T. evansi	cattle, equines, camelids, buffalo, sheep, goats, pigs, dogs	tabanid flies	mechanical	Africa, Asia, S.America
T. equinum #	equines, dogs, cattle	tabanid flies, vampire bats	mechanical	S.America
T. equiperdum	equines	(venereal transmission)	via coitus	S.Europe, Asia, N.Africa

* Within the species *T. brucei* there are two other sub-species which cause sleeping sickness in man, *T.b. gambiense* and *T.b. rhodesiense.*

T. equinum is generally regarded as a New World variant of *T. evansi* (Brown *et al* 1990).

Table 2.1 : Salivarian trypanosomes of domestic animals; their definitive hosts, vectors, mode of transmission and distribution (adapted from Smyth, 1994).

Mammalian trypanosomes are divided into two sections *Stercoraria* and *Salivaria* according to their mode of transmission. Stercorarian trypanosomes are passed to the mammalian host in the faeces of the fly vector, whereas salivarian trypanosomes are transmitted in fly saliva during feeding. This review will focus on the salivarian trypanosomes and their importance in draught animals. The nine species of salivarian trypanosome which infect domestic animals are listed in Table 2.1 together with their vectors, preferred host species and geographic distribution. The primary species afflicting draught animals are *T. vivax, T. congolense, T. brucei and T. evansi*, the first three of which are generally cyclically transmitted by the tsetse fly (*Glossina* spp.). *T. evansi* is mechanically transmitted by tabanids and other biting flies. Mechanical transmission of trypanosomes by tsetse flies is also possible (Itard, 1981), although seldom seen in the field (Brown, Hunter and Luckins, 1990).

2.3.2 Tsetse transmitted trypanosomosis

Tsetse transmitted trypanosomosis is confined exclusively to sub-Saharan Africa, where it is a major constraint on livestock productivity and agricultural development in general (Connor, 1992; Itty, 1992). It has been estimated that 60 million cattle in 36 countries across the continent face a serious risk of contracting the disease (Chadega, 1994; FAO, 1994). Land use is severely restricted across an area of 10 million km^2 straddling the equator from 15°N to 20°S, which is infested by the tsetse fly vector (Sileghem, Flynn, Darji, De Baetselier and Naessens, 1994). Different species of tsetse fly differ in their ability to transmit pathogenic trypanosomes, however the more efficient vectors such as *G. morsitans* completely preclude the keeping of domestic livestock in certain areas (Brown *et al* 1990). Consequently Finelle (1974) noted that in addition to increased morbidity and mortality amongst domestic livestock, one of the indirect consequences of trypanosomosis is the absence

or near absence of mixed farming systems and a reduction in overall farm productivity in some regions, due to a shortage of animal traction.

Fly distribution is determined by climate and the resulting vegetation (Itty, 1992). Itard (1981) suggested that suitable ecoclimates for tsetse are areas of woodland in regions with an annual rainfall in excess of 1000 mm *p.a.* and average annual temperatures between 20 and 32°C; land areas which but for the tsetse fly might otherwise be ideal for cultivation or grazing (MacLennan, 1981). Within these areas different fly species inhabit different microclimates, thus there are recognised riverine, savanna and forest dwelling species (Itard, 1981). Although tsetse fly infestations are normally associated with areas of wilderness they can also inhabit cultivated areas where there is sufficient vegetation to rest up between feeds. MacLennan (1981) cited several of examples of tsetse infestations in arable plantings.

There are a number of safe therapeutic and prophylactic drugs available which have allowed cattle to be kept and worked in tsetse infested areas, however the drugs are expensive (Murray, Morrison and Whitelaw, 1982) and furthermore no new ones have been developed for 30 years, consequently drug resistance is becoming an increasing problem (Clausen, Sidibe, Kabore and Bauer, 1992; Dolan, Stephenson, Alushula and Okech, 1992; Fox *et al* 1993; Sutherland, Codjia, Moloo, Holmes and Peregrine, 1992). A wide variety of vector control techniques have also been tried, to limit the incidence of the disease, including, bush clearance, eradication of wild game, biological control using sterile flies, insecticide spraying of vegetation, baited traps and insecticide treatment of cattle. These techniques have been reviewed in detail by Itard (1981) and Singh (1995). Tsetse control has been effective in some regions, although as Murray *et al* (1982) commented the cost is high, and constant monitoring and surveillance are required to avoid reinfestation. Trypanotolerant cattle offer a sustainable alternative to drugs and vector control.

Transmission of the disease with particular reference to draught animals

Tsetse fly biology has been extensively studied in the search for suitable control methods. Detailed below are some points pertinent to the transmission of trypanosomosis in draught animals.

Adult tsetse flies feed entirely on the blood of vertebrates. The flies themselves become infected with trypanosomes when they feed on an animal that is already infected. Infection has no detrimental effect on the fly (Stephen, 1986). After ingestion the trypanosomes develop within the fly for between 5 and 35 days before infective metacyclic trypanosomes are produced, whereupon the fly is capable of transmitting the parasite to a new mammalian host (Brown *et al* 1990). Flies can harbour mixed infections of different trypanosome species and usually remain infective for life (Stephen, 1986) although infection rates and transmission rates vary considerably with both fly and trypanosome species. Males flies may feed as often as every second day depending upon species, climate, sexual activity and host availability. Females feed more erratically depending upon their reproductive state but take larger blood meals. Itard (1981) commented that although infection rates in wild flies appear low, the highest rate being about 19%, serious epidemics of trypanosomosis can still occur because of the frequency of feeding and the fact that flies remain infected for life. The average life-span of an adult female is 60 to 90 days during which time she will produce a maximum of approximately 10 progeny. Males live for only 30 days (Itard, 1981), however a single mating is sufficient to ensure that the female fly remains fertile for life (Jordan, 1986).

Although draught animals are often kept and worked singly or in small numbers seldom meeting other animals, this comparative isolation does not provide the protection against trypanosomosis that it might afford against a contagious disease because of the mobility of the insect vectors. MacLennan (1981) stated that no livestock project is safe from infection if there is a primary focus of *G. morsitans*, one

of the savanna dwelling fly species, within a radius of 16 km. Riverine tsetse flies persist in natural vegetation even in areas of very heavy human settlement (MacLennan, 1981) and are known to travel up to 20 km along watercourses (Itard, 1981).

Tsetse flies are most active during the rainy season when warm humid conditions allow them to fly further and live longer than at other times of year (Itard, 1981). There is also an increase in fly infection rates at this time of year, caused by shorter trypanosome development cycles and an increase in trypanosome infectivity again associated with the climatic conditions (Itard, 1981). This increase in fly activity and infection rates coincides with the start of the cultivation season for draught animals.

Fly feeding activity particularly amongst savanna dwelling flies often coincides with the times of day when farmers are most likely to be working their animals. During the hot season flies hunt for brief periods in early morning and late afternoon, avoiding the midday heat (Pilson and Pilson, 1967). During the rains hunting activity is more uniformly distributed throughout the day. Forest flies feed from mid-morning until early evening regardless of season (Itard, 1981). Riverine flies pose a particular threat when animals are brought to water.

Tsetse flies seek out their host targets using a combination of olefactory and visual stimuli, the former are effective over distances of 90m, the latter at up to 50m (Vale, 1977). They prefer to hunt in open areas where flying is easy (Itard, 1981), consequently animals cultivating fields probably present ideal targets. Some fly species are particularly attracted to moving targets (Vale, 1977) others are more strongly attracted by odour (Willemse and Takken, 1994). Host complacency is important in determining whether a fly is able to feed successfully once it has landed on an animal (Fiske, 1920). Most animals if they are aware of a tsetse fly alighting on

them will try to dislodge it before it is able to feed. Animals harnessed to implements may be less able to do so, although if the driver sees the fly he is likely to remove it.

The indigenous mammals of Africa are the natural hosts of the three main tsetse transmitted trypanosome species that afflict draught animals; *T. vivax, T. congolense* and *T. brucei.* Wild animals do not generally suffer from pathogenic disease unless stressed, but because of high infection rates they are important reservoir hosts from which flies can acquire infections which are subsequently passed on to domestic stock (Itard, 1981). Thus the presence or absence of other cattle in an area may have little bearing on the likelihood of the draught animals becoming infected with trypanosomosis. Although the feeding habits of tsetse are generally quite specific, with different species having different preferred food hosts, Stephen (1986) noted that in the absence of their preferred hosts flies are cosmopolitan feeders seeking a blood meal wherever it is available. The feeding habits of tsetse flies have been reviewed in detail by Weitz (1963) and more recently by Itard (1981) and Stephen (1986).

The effects of the parasite on the host mammal

Tsetse transmitted trypanosomosis is usually regarded as a chronic disorder in domestic livestock (Itard, 1981), however the severity and duration of infections vary considerably depending upon a number of factors including the host species and breed and the species and strain (serodeme) of the infecting trypanosome (Stephen, 1986) (Table 2.2). The disease is characterised by fluctuating waves of parasitaemia and coincident pyrexia, accompanied by a progressive anaemia (Stephen, 1986). Some animals develop fulminating parasitaemias and die very quickly often within a fortnight of infection. More commonly animals develop chronic infections which persist for months with few obvious symptoms other than a progressive loss of body condition. Trypanosomosis is not always fatal, although without treatment mortality

is often high (Hall, 1988). Animals, particularly those from certain breeds regarded as trypanotolerant, infected with small numbers of trypanosomes from strains of low virulence can recover within a few weeks given good nutrition and management. Occasionally animals self cure after more severe infections lasting several months (Brown *et al* 1990).

Animal	***T. vivax***	***T. congolense***	***T. brucei***	***T. evansi***
Zebu cattle	***	***	**	**
W.African dwarf cattle	**	**	**	?
Horses	* or ***	*	***	***
Donkeys	* or ***	*	*	*
Camels	* or ***	*	***	***
Swamp buffalo	n/a	n/a	n/a	**

* = mild, ** = chronic, *** = acute, ? = not known, n/a = not applicable

Table 2.2 : Pathogenicity of the major tsetse and tabanid transmitted trypanosomes in animals used for draught power (adapted from Stephen, 1986).

An animal infected by a feeding tsetse fly will develop a patent parasitaemia within 3 to 16 days. The duration of prepatency depends upon the species and strain of the infecting trypanosomes and on the number of trypanosomes which originally entered the blood (Brown *et al* 1990). Following infection there is normally an acute phase of the disease when parasite numbers within the blood rise and fall rapidly causing a relapsing high parasitaemia and fever with a developing anaemia. The dynamics of trypanosome-induced anaemia and the mechanisms behind it have been

reviewed in detail by Murray and Dexter (1988). The acute phase of infection can last up to 12 weeks in cattle (Brown *et al* 1990). If the animal survives the disease will enter a chronic phase when the parasitaemia is low and trypanosomes are often undetectable in blood samples. This chronic phase can persist for months even years during which time the animal becomes progressively more anaemic, emaciated and lethargic until eventually it dies of a combination of myocardial damage and anaemia. Anaemia, emaciation and lethargy are all of particular concern in working animals; anaemia because it reduces the oxygen carrying capacity of the blood and consequently may limit aerobic capacity; emaciation because the power an animal is able to generate is related to its body weight; and lethargy because it makes animals less workable. MacLennan (1981) noted that draught animals often die of heart failure if they are worked whilst suffering from trypanosomosis because of the myocarditis and anaemia. Stressors such as prolonged trekking, work, pregnancy, lactation, malnutrition or intercurrent disease can also trigger a recurrence of acute disease in chronically infected animals.

Other symptoms of trypanosomosis include oedema (more common in horses than in cattle), splenomegaly, polyadenitis, nervous disorders and motor incoordination including paresis of the hind limbs, pica, eye disorders, diarrhoea and emaciation leading to cachexia (Itard, 1981). A number of these symptoms could prevent an animal from working.

The characteristic cyclical rise and fall in parasitaemia observed in trypanosomal infections is caused by a mechanism known as antigenic switching or antigenic variation employed by the parasite to evade the host immune system. This mechanism involves spontaneous changes in surface antigens known as variant surface glycoproteins (VSGs) which protect the plasma membrane of the trypanosome (Smyth, 1994). These changes occur every few days rendering specific antibodies produced by the host immune system against the VSGs ineffective because of the

appearance of new variant antigen types (VATs) (Donelson and Turner, 1985). The number of trypanosomes in the blood of the host thus increases as the parasites multiply and then decreases as the host's immune system develops specific antibodies against the trypanosomal VSGs expressed until the surviving trypanosomes start to develop new VATs and parasite numbers rise again. The dynamics of antigenic variation are described in detail by Barry and Turner (1992). The range or repertoire of VATs that a single serodeme can express is large, possibly as many as several hundred (Brown *et al* 1990). Different serodemes have different repertoires (Smyth, 1994). Consequently although cattle may acquire some immunity to a particular serodeme following infection, they remain vulnerable to challenge with heterologous serodemes (Brown *et al* 1990).

2.3.3 *Trypanosoma evansi*

T. evansi is widespread, occurring in the Middle and Far East, Asia, Central and South America and parts of Africa on the northern edge of the tsetse fly belt. Trypanosomosis caused by *T. evansi* affects a wide variety of domestic ruminants (Table 2.1), a number of which are used to provide farm power or transport. This review focuses specifically on the disease in the Asia where it is known as surra. Surra is an important disease of large ruminants throughout S.E. Asia (Dieleman, 1986). In Indonesia it is regarded as one of the most important livestock diseases in the country (Adiwinata and Dachlan, 1969; Dieleman, 1986; Winrock International Institute for Agricultural Development, 1986) which in 1984 was estimated to cost the country up to US$ 28 million p.a. in lost productivity (Directorat Jenderal Peternakan, 1984) although the figures were not substantiated.

In areas where the parasite is endemic, surra is generally regarded as a chronic often sub-clinical disease of cattle and buffalo causing low mortality (Dieleman, 1986), but significant economic loss through reduced productivity (Payne, 1989). In

horses the disease is acute and invariably fatal (Bakkar, 1930; Molyneux and Ashford, 1983) unless trypanocidal drugs are administered (Payne, 1989). Epidemics of surra do occasionally occur in cattle and buffalo resulting in high morbidity and mortality; for example in late 1968 an epidemic in Central Java lasting six months killed 1,870 buffalo, 783 cattle and 22 horses (Adiwinata and Dachlan, 1969). Hoare (1972) suggested that such outbreaks of clinical surra may be triggered by the importation of naive animals into an area where the pathogen is endemic, or alternatively by the introduction of a new strain of the parasite into an area via a carrier animal. Stress has also been associated with severe outbreaks of clinical disease in cattle and buffalo (Bakkar, 1930; Doeve, 1917; Wells, 1981).

Transmission of the disease with reference to draught animals

Unlike tsetse flies which are confined to sub-Saharan Africa, vectors of *T. evansi* are found across the tropics and sub-tropics in a wide range of climates and vegetation types. The disease is mechanically transmitted by hematophagous biting flies from several genera the most important of which are the tabanids (Brown *et al* 1990). The principles of disease transmission do not vary significantly between vector species. Because of the variety of potential vectors and the diversity of their habitats, controlling surra by eliminating the flies is not regarded as a practical proposition (Dieleman, 1986).

In contrast to cyclical transmission, with mechanical transmission the vector itself does not become infected with trypanosomes but merely transfers the parasites from one mammalian host to another via its mouthparts whilst feeding. When a fly feeds on an infected animal if the parasitaemia in the host is sufficiently high the fly's mouthparts become contaminated with infective blood. If that fly then subsequently feeds on another animal within a short space of time, it will transmit the disease, if not the trypanosomes die. Work by Nieschulz, (1928 and 1930) cited by Dieleman

(1986) has demonstrated that tabanids can transmit surra for up to six hours after an infective feed, although as Molyneux and Ashford (1983) noted the shorter the interval between feeds or attempted feeds, the greater the chance of transmission. One tabanid can infect three animals in succession (Nieschulz and Ponto, 1927, cited by Dieleman, 1986). Other fly species are generally considered inefficient vectors of surra because of the very limited survival time of trypanosomes on their smaller mouthparts and because of their inability to inflict the deep puncture wounds required for easy infection (Payne, 1989). Parasite survival time on the vector's mouthparts becomes less critical at higher host stocking densities.

Only the adult females tabanids feed on mammalian blood, males live on flowers and are harmless (Itard, 1981). Both sexes of the other *T. evansi* vector species are however haematophagous, like the tsetse fly.

Tabanids are particularly effective vectors because they are aggressive and persistent feeders, annoying the host which then attempts to dislodge the fly (Brown *et al* 1990). Dislodged flies readily seek new hosts so that several animals may be bitten in quick succession. Keeping or working animals in close proximity therefore encourages transmission. This was recognised as early as 1917 by Doeve (1917) who recommended that buffalo showing clinical signs of surra should not be worked alongside healthy animals.

Transmission is also affected by the degree of parasitaemia in the animal on which the fly feeds (Brown *et al* 1990). Flies feeding on animals with high parasitaemias are more likely to become contaminated with infective blood and thus spread the disease than those feeding on animals with lower parasitaemias. Payne (1989) noted that horses provide good short term foci of infection as they generally develop acute infections with high parasitaemias.

Tabanids have a flight range of 1 - 3 km (Molyneux and Ashford, 1983) and hunt by sight (Itard, 1981), feeding on average every other day (Dieleman, 1986). They are particularly attracted by larger animals and animals in motion, such as

working cattle or buffalo. In hot climates tabanids feed in the early morning and at dusk, avoiding the heat of the day (Leese, 1909), in cooler climes they are often active throughout the day (Itard, 1981).

Surra appears to be maintained by transmission cycles within domestic livestock and in contrast to tsetse transmitted trypanosomosis wild animal hosts are not regarded as significant in the epidemiology of the disease (Brown *et al* 1990). Reviewing the evidence from a number of experimental infections Mahmoud and Gray (1980) suggested that donkeys, cattle, sheep and goats may all act as reservoir hosts for surra.

In a number of countries in Africa and Asia the prevalence of surra increases during and immediately after the rainy season when fly numbers rise in response to the wet and humid conditions (Stephen, 1986). Most of the vector fly species require damp or wet breeding sites (Itard, 1981). This seasonal increase in the incidence of surra occurs at precisely the time when draught animals are in greatest demand for cultivation work. In a survey of cattle, buffalo and horses in Indonesia, Payne, Sukanto, Djauhari, Partoutomo, Wilson, Jones, Boid and Luckins (1991) discovered that surra infection rates were correlated with annual rainfall; areas with higher annual rainfall tended to have higher surra infection rates than areas of lower rainfall. Similar correlations between rainfall fly numbers and the incidence of surra have been reported in Sudan and India (Mahmoud and Gray, 1980; Stephen, 1986). In areas with permanent water sources the disease is less seasonal. Payne (1989) suggested that wetland rice cultivation as practised extensively in Indonesia provides an ideal habitat for tabanids and the transmission of surra throughout the year. Draught animals, particularly buffalo are widely used in such systems.

The effects of the parasite on the host

In common with the tsetse transmitted trypanosomes, strains or serodemes of *T. evansi* from different geographical locations differ significantly in their virulence and pathogenicity in domestic livestock. The symptoms of surra are very similar to those observed with tsetse-transmitted trypanosomes, namely fluctuating parasitaemia and fever with a progressive anaemia. As already noted these symptoms can have a negative effect on work performance (section 2.3.2). Clinical signs however vary considerably depending upon the animal infected.

After an incubation period of 8 to 10 days, horses develop a transient high fever with trypanosomes appearing in peripheral blood 1 to 2 days later. High parasitaemias are accompanied by a loss of appetite, and general weakness together with an elevated respiration and pulse rate. Oedema, larchrymation and a dull staring coat are also common features of infection in horses (Itard, 1981). Although appetite generally recovers after a few days there is a progressive emaciation. Within two weeks of infection parasites may enter the central nervous system causing paralysis of the hindquarters. Mortality is extremely high, often approaching 100% (Bakkar, 1930; Stephen, 1986).

Buffalo and cattle can suffer from acute surra with similar symptoms to horses, however in enzootic areas they generally develop chronic infections (Dieleman, 1986) which are often hard to detect by parasitological means (Bakkar, 1930). These can last several months at a time with few clinical signs beyond a transient depression in food intake and progressive emaciation. Emaciation may ultimately lead to death through exhaustion, particularly in working animals. With rest and good nutrition however cattle and buffalo can overcome infection even without treatment (Doeve, 1917). After protracted infections some animals become symptomless carriers acting as reservoirs of infection (Mahmoud and Gray, 1980). Mortality rates in endemic areas are usually low (Bakkar, 1930). In Indonesia Adiwinata (1957) reported a mortality rate of 3.2% for cattle and 2.8% for buffalo,

however both the incidence of the disease and the outcome of infections can be adversely affected by stress. Stressors such as malnutrition, work, pregnancy and exposure to adverse weather conditions have been reported as contributory factors in severe outbreaks of surra in a number of different countries (Doeve, 1917; Löhr *et al*, 1985; Löhr *et al*, 1986; Wells, 1981). Surra during pregnancy is reputed to cause abortions in buffalo in Thailand (Löhr *et al*, 1985).

2.3.4 Immune responses to infection

Humoral immune responses are considered to be the main method by which animals seek to control trypanosomal infections (Murray *et al*, 1982). The introduction of trypanosomes into the mammalian host triggers a rapid and strong antibody response. This response however differs from the typical primary humoral response of mammals against most pathogens, in which initial increases in IgM antibodies are subsequently replaced by the synthesis of IgG. In trypanosome infected hosts IgM production is greatly enhanced and prolonged. In cattle IgM titres have been reported to increase by 8 to 10 fold within 14 to 21 days of a trypanosome infection (Clarkson, Penhale and McKenna, 1975; Kobayashi and Tizard, 1976; Luckins, 1972 and 1976) with titres remaining high for several months after infection or until the animals were treated with trypanocides. Work by Clarkson *et al* (1975) and more recently by Luckins and Mehlitz (1978) has however demonstrated that IgM increases can vary markedly between individuals. IgG concentrations can double between days 20 and 60 after infection (Clarkson and Penhale, 1973; Luckins and Mehlitz, 1978). More detailed analysis of immunoglobulin subclasses has suggested that elevated IgG titres are due primarily to an increase in IgG_1. Kobayashi and Tizard (1976) demonstrated that IgG_1 titres in european calves infected with *T. congolense* increased by 2.5 fold within 7 weeks whereas IgG_2 only increased 1.6

times over the same period. IgA does not appear to increase in response to trypanosomal infection (Clarkson and Penhale, 1973).

Many of these immunoglobulins were originally assumed not to be parasite-specific (Freeman, Smithers, Targett and Walker, 1970; Houba, Brown and Allison, 1969), however work by Musoke, Nantulya, Barbet, Kironde and McGuire (1981) and Masake, Musoke and Nantulya (1983) has since indicated that much of the IgG and IgM produced is specific for the VSGs of the infecting trypanosomes. Recurrent peaks of antibody activity against the VSG's of the infecting trypanosome population have been found in cattle infected with *T. brucei* (Nantulya, Musoke, Barbet and Roelants, 1979) and *T. congolense* (Masake *et al* 1983). Nantulya *et al* 1979 hypothesised that these peaks were caused by the reappearance of parasites with the same or similar VSGs.

Much early work concentrated on humoral aspects of immunity including immunoglobulins and protective antibodies, however more recently it has become clear that T cells also play an important part in the immune response to trypanosomes. Although T cells do not have a direct effect on trypanosome destruction, they have an regulatory role in the induction of immunity which is important in the outcome of the disease. This aspect of immunity is complex, but is outside the scope of this thesis. It will not be discussed in detail here, however it is comprehensively reviewed by Sileghem *et al* (1994).

2.3.5 Trypanotolerance

Certain West and Central African breeds of humpless, taurine, dwarf cattle such as the N'Dama and Muturu are regarded as trypanotolerant being able to survive and reproduce in areas of tsetse challenge, where without the aid of chemotherapy other cattle rapidly succumb to trypanosomosis (Chandler, 1952; 1958; Desowitz, 1959; Stephen, 1966). Although it is a universally recognised term, Murray,

Morrison, Murray, Clifford and Trail (1979) commented that trypanotolerance is a misnomer, since these animals can become infected with trypanosomes, with adverse effects and they do develop immune responses to infection. The same authors suggested that trypanotolerance is more accurately described as a reduced susceptibility to the pathogenic effects of the disease.

Trypanotolerant cattle display other useful traits in addition to resistance to trypanosomosis. They are reported to be resistant to streptothricosis (Coleman, 1967; Oduye and Okunaiya, 1971; Stewart, 1937), to ticks and tick-borne diseases including anaplasmosis, babesiosis and heartwater (Epstein, 1971) and to some extent to helminthiasis (Ilemobade cited by Murray, 1988). They are also well adapted to heat stress and water restriction (Murray and Trail, 1983).

Trypanotolerance is an innate, heritable, genetic trait (Murray, 1988), which results in a superior ability to control parasitaemia and the development of anaemia when infected (Murray, Murray, Wallace, Morrison and McIntyre, 1977; Paling, Moloo and Scott, 1988). These characteristics have been demonstrated following both needle challenge (Dwinger *et al*, 1992) and natural infection by tsetse flies (Murray, Clifford, Gettinby, Snow and McIntyre, 1981) with single and mixed trypanosome species infections (Murray *et al*, 1977b). Trypanotolerant cattle are able to control, reduce and on occasion eliminate trypanosome infections, probably due to a superior anti-trypanosome immune response (Murray *et al* 1982), however the exact mechanisms involved are not known (Authié, Duvallet, Robertson and Williams, 1993a).

Acquired immunity contributes significantly to the resistance achieved under natural challenge (Authié, 1994), as has been confirmed in a number of studies (for example by Desowitz, 1959; Saror, Ilemobade and Nuru, 1981; Touré, Gueyé, Séyé, Ba and Mané, 1978). As Murray *et al* (1982) commented however, this is not confined to trypanotolerant breeds.

Trypanotolerance is a relative rather than absolute trait (Leak, d'Ieteren and Rowlands, 1994). Trypanotolerant animals can show clinical symptoms of disease and on occasion tolerance may breakdown leading to severe and even fatal trypanosomosis. Breakdowns have been attributed to high tsetse challenge and stress (Murray *et al* 1982). A range of stress factors has been incriminated in increasing susceptibility to trypanosomiasis including malnutrition, overwork, intercurrent disease, (MacLennan, 1970) pregnancy, lactation (Murray, 1988) and even repeated bleeding (Stewart, 1951).

There has been a recent resurgence of interest in trypanotolerant cattle and their ability to resist trypanosomosis without chemotherapy, because of the limitations of the current methods of controlling the disease (Dwinger *et al*, 1992). As indicated in section 2.3.2, chemotherapy for the protection and treatment of livestock is expensive and drug resistance is becoming an increasing problem. Vector control although it has been successful in some areas (MacLennan, 1981) has severe limitations, which include cost and the need for constant vigilance to avoid reinvasion by the tsetse fly (Murray, Trail and d'Ieteren, 1990). Furthermore the widespread use of insecticides is now environmentally unacceptable (Singh, 1995). An effective vaccine for protecting stock is not currently available and does not look likely in the near future (Dwinger *et al* 1992). Starkey (1982) commented that although they are not ideal draught animals due to their small size, trypanotolerant cattle can play an important role in the provision of farm power where tsetse flies are a threat to other breeds. Whilst this is true, in some circumstances smaller draught animals may be more appropriate than larger ones. Clearly trypanotolerance is not absolute and the implications of stressors such as work and level of feeding on the ability of these animals to resist the disease warrants further investigation and understanding so that the attributes of the animals can be realised.

3. GENERAL METHODS

This chapter contains details of methods common to several experiments.

3.1 Work output & energy expenditure of working animals

3.1.1 Measurement of draught forces, distance travelled & work output

In the two studies in which animals pulled sledges round a track (Chapters 4 and 6) the work output (kJ) of one team of animals (nominally the control team) was monitored throughout the study using an ergometer (Lawrence and Pearson, 1985). This ergometer comprised a load cell (Type F241, range 0 - 3000 N, Novatech Ltd, St. Leonards-on-Sea, UK) attached between the sledge draw chain and the animal yoke which measured draught force (N), an odometer wheel trailed behind the sledge which measured distance travelled (m) and in the Gambian trial an integrate and display unit mounted on the sledge which integrated force and distance, displaying work done and the distance travelled on liquid crystal digital displays. Readings from the displays were manually recorded after each circuit of the track. In the study carried out in Indonesia the integrate and display unit was replaced by a data logger unit (Pearson et al, 1989) which after integrating force and distance stored all incoming data onto a Husky microcomputer (Husky Computers Ltd., Coventry, UK). This more sophisticated unit was also used to record the elapsed work time and the rectal temperatures of the two animals to which the ergometer was attached, whilst they were working. After work each day the data from the Husky was downloaded

onto another computer and imported into a spreadsheet software package for analysis (Excel 5.0, Microsoft Corporation, USA).

3.1.2 Average draught force

The daily work output of each of the teams not monitored by ergometer was estimated from the distance travelled by that team on that day multiplied by the average draught force (ADF) required by the team to pull its sledge as measured on a specific ADF test day before the experiment but with a correction factor applied to compensate for variations in draught force caused by track surface conditions.

On the ADF test day the ergometer was attached to each team in turn, including the control team. For each team a number of draught force measurements were made as the animals pulled their sledge over randomly chosen sections of the track to be used for the study. The mean of the measurements was taken as the team ADF.

In the Indonesian study to calculate the work output of a team on a particular day during the experiment, the team ADF was corrected by multiplying it by the ADF of the control team on that particular day divided by the ADF of the control team recorded on the test day. In the Gambian study where track surface conditions did not change over the course of the experiment, the correction factor used was the mean ADF of the control team over the experiment divided by the ADF of the control team recorded on the test day.

3.1.3 Calculating energy expenditure for work

Animal net energy (NE) expenditures for work were calculated using the factorial method developed by Lawrence (1985) as follows. As the animals were worked in pairs on a yoke, each animal was assumed to do half of the work and carry half of the weight of the yoke:

$$Energy_{work} = Energy_{walking} + Energy_{carrying\ loads} + Energy_{pulling\ loads}$$

$$E = AFM + BFL + \left(\frac{W}{C}\right)$$

Equation 3.1

E = Net energy (NE) used for work (kJ)

F = distance travelled (km)

M = animal liveweight (kg)

L = load carried (kg)

W = work done whilst pulling the load (kJ)

A = energy used to move 1 kg of body weight 1 m horizontally (J)

B = energy used to move 1 kg of applied load 1 m horizontally (J)

C = efficiency of doing mechanical work

Work outputs and energy expenditures for work were expressed per animal and also per unit of metabolic liveweight ($LW^{0.75}$) to allow comparison with other draught animal studies and with feed energy inputs, which if calculated using the ME system according to the Agricultural Research Council (ARC) recommendations (1980) are based on animals metabolic liveweights.

ME requirements for work were calculated by dividing NE expenditures by the efficiency of utilisation of ME for maintenance (k_m) (ARC, 1980):

$$k_m = 0.35q_m + 0.503$$

Equation 3.2

q_m = metabolisability of the ration for maintenance (ME/GE).

The efficiency of utilisation of ME for work was assumed to be the same as that for maintenance (Lawrence and Becker, 1995).

3.2 Enzyme-linked immunosorbent assays (ELISA)

Two types of ELISA were used in these studies, an indirect antibody ELISA and an indirect double antibody sandwich ELISA. The former was used to measure sheep serum antibody responses to two activating antigens (Chapter 5), also to test for the presence of variant specific anti-trypanosomal antibodies in cattle sera and following infection to measure amounts of anti-trypanosomal IgM and total IgG in sera (Chapter 6). Indirect double antibody sandwich assays were used to measure amounts of parasite specific IgG_1 and IgG_2 in the cattle sera after infection. For the sheep assays crude preparations of the activating antigens were used as the ELISA antigens, for the cattle assays crude trypanosome extracts were used.

3.2.1 Protocol used for indirect antibody ELISA

Flat bottomed 96 well micro-titre plates (Immulon 1®, Dynatech Ltd, USA.) were coated with the appropriate antigen diluted in freshly prepared 0.05M carbonate-bicarbonate buffer, pH 9.6 (Sigma Chemical Co. Ltd., Poole, UK), in 100 µl volumes per well and left to incubate overnight at 4°C. The following morning excess antigen was discarded and the plates were washed three times with a solution of PBS (pH 7.4) containing 0.05% Tween 20 (PBS-T) (Appendix 1), with a 3 minute soak in each wash cycle. 100 µl volumes of an appropriate dilution of the test sera in PBS-T, were placed in each well and the plates then incubated at 37°C for 30 minutes. After incubation the serum was discarded and the plates were washed three times as above.

Thereafter 100 µl volumes of horseradish peroxidase conjugated antiglobulin were added to all wells, diluted according to the manufacturer's recommendations in

PBS-T. The plates were then incubated at 37°C for a further 30 minutes, the excess conjugate was thrown out and the plates were given a final three washes with PBS-T.

Whilst the plates were being washed, fresh 0.05 M phosphate-citrate buffer (pH 5.0) with 0.014% urea hydrogen peroxide was prepared (Sigma Chemical Co. Ltd.). The chromogen 3,3′ 5,5′-tetramethylbenzidine dihydrochloride (TMB, Sigma Chemical Co. Ltd.) was dissolved in the buffer (1 mg of substrate in 10 ml of buffer) immediately prior to use. 100 µl of this substrate solution was then pipetted into each well on the plate, prior to a final 15 minute incubation at 37°C.

The reaction was stopped by the addition of 50 µl volumes of 2M sulphuric acid to all wells and the OD of the well contents measured at a wavelength of 450 nm using a plate reading spectrophotometer (Titertek Multiscan, Labsystems Ltd., Basingstoke, UK). Data from the spectrophotometer was down-loaded directly to a computer and subsequently imported into a spreadsheet analysis package (Excel 5.0, Microsoft Corporation, USA).

3.2.2 Protocol used for indirect double antibody sandwich ELISA

The methodology adopted for indirect double antibody sandwich assays was the same as that used for the indirect antibody ELISA, but with the addition of an extra step. After the serum had been discarded and the plate washed three times, 100 µl of diluted class-specific monoclonal antibody was added to each well and the plate was incubated at 37°C for 30 minutes before being washed three times as above. Thereafter 100 µl volumes of horseradish peroxidase conjugated antiglobulin were added to each well as per the indirect antibody ELISA protocol.

3.2.3 Establishing the reaction parameters for individual assays

To establish working dilutions for the antigen and sera for each ELISA, a series of chequerboard titrations were carried out, cross-testing serial dilutions of the coating antigen against known positive and negative control sera. Both the coating antigens and the test sera were tested as doubling dilution series, although the starting point for each varied depending upon the assay. The conjugates for the assays were used at the manufacturers' recommended dilutions, as were the class-specific anti-bovine monoclonal antibodies for the double antibody sandwich ELISA's. The plate layout used for these titrations is illustrated in Figure 3.1. A number of wells on each plate were filled with washing buffer (PBS-T), instead of serum, as blanks to estimate the background absorption of the wells.

	Antigen coating dilutions												
	1:500			1:1000			1:2000			1:4000			
	1	2	3	4	5	6	7	8	9	10	11	12	Serum dilution
A	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:200
B	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:200
C	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:400
D	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:400
E	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:800
F	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:800
G	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:1600
H	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	PBS	+ve	−ve	1:1600

+ve Well containing positive serum sample
−ve Well containing negative serum sample
PBS Well containing PBS-T only, no serum

Figure 3.1 : Example of a chequer-board microtitre plate layout used to determine optimum antigen and sera dilutions for ELISA's.

Evaluation of chequerboard assays

Optimal dilutions for each assay were taken as those which resulted in minimal background OD's in the PBS-T wells and the negative serum control wells, but with binding ratios of at least 5:1 (Positive control serum OD : negative control serum OD), (Voller *et al*, 1979). However an important consideration when deciding appropriate dilutions for the assays was also the need to conserve precious reagents, particularly the trypanosomal antigens.

3.2.4 Procedures used to minimise variations within an ELISA

Nine procedures were adopted to minimise the effects of intra and inter plate variations on each assay:

- Pipette calibrations were checked before each assay series. The same pipettes and make of pipette tips were used throughout.
- Coating antigen and control sera were aliquoted into the volumes required for a single day's testing prior to each assay, to avoid excessive freezing and thawing of reagents and samples.
- Where very small volumes (<4 µl) of monoclonal antibody and conjugate were required, intermediate dilutions were prepared, aliquoted and frozen to minimise errors inherent in pipetting very small volumes.
- Each assay was completed using a single batch of plates, monoclonal antibodies and conjugate to avoid any batch to batch variation.
- All buffers were freshly prepared and checked to ensure they were at the correct pH before use (Wright and Nielsen, 1988).
- Incubation times and temperatures were strictly adhered to.

- All sera were tested in duplicate, with the mean OD of the two wells being the final result. In all assays other than those used to measure trypanosomal IgM, if the OD's of duplicate wells differed by more than 10% of the anti-log10 of the larger OD the result was considered unsatisfactory (Scott, 1994) and where possible the serum sample was retested. 20% of the anti-log10 was used as the cut off point for the assay measuring trypanosomal IgM. Logarithmic transformations were used to avoid being unduly rigorous on smaller values, which would have been a problem had the lower limit been defined merely as a percentage of the larger OD (Appendix 2).
- Plate to plate variations in the OD of positive and negative control samples were monitored. Plates with mean control values differing from the overall means for the assay by more than two standard deviations were considered abnormal (Appendices 3 to 7). Data from such plates was excluded from inter-plate comparisons.
- Where possible the outside wells on the plates were not used for sera but were filled with PBS-Tween to reduce possible edge effects (Kemeny and Chantler, 1988).

3.3 Statistics

The distribution-dependency of data was tested using a Bartlett's test (Sokal and Rohlf, 1981) and a Shapiro-Wilks test (Shapiro and Wilks, 1965) with a normality plot. Data were assumed to be normally distributed and were analysed using parametric tests if $P > 0.05$ in both tests. Failing this non-parametric analyses were used.

Normally distributed data are summarised as means ± 1 standard error (s.e.) non-parametric data as medians with semi-interquartile ranges (s.i.r.). Throughout this thesis the following notation is used to denote varying levels of statistical significance; $P > 0.05$, not significant 'ns'; $P \leq 0.05$, significant '*'; $P \leq 0.01$, highly significant '**'; and $P \leq 0.001$, very highly significant '***'.

A range of statistical tests were used for data analysis, these are detailed in individual chapters. When an analysis of variance (ANOVA) of normally distributed data showed significant differences, the differences between individual means were tested by least significant difference (LSD) (Sokal and Rohlf, 1981). When a Kruskal-Wallis (KW) test of non-parametric data showed significant differences between medians a multiple comparison test (Siegal and Castellan, 1988) was used to determine which of the medians were significantly different (Appendix 8).

Unless otherwise specified, all results expressed as percentages were transformed using a standard angular transformation for percentage data (arcsine $\sqrt{\text{percentage}}$) (Sokal and Rohlf, 1981) before being subjected to any parametric analyses.

Statistical analysis of the results was performed using Minitab (Release. 10.2, Minitab Inc., USA) and Excel 5.0 (Microsoft Corp., USA) computer software packages.

4. THE EFFECTS OF *TRYPANOSOMA EVANSI* ON THE WORK OUTPUT OF SWAMP BUFFALO IN INDONESIA

4.1 Introduction

Draught animal power underpins the economies of most of the states of south and east Asia (Campbell, 1993). In Indonesia cattle and buffalo are estimated to provide 70% of the draught power required for cultivation and transportation in agriculture (Winrock International Institute for Agricultural Development, 1986) (although it is not specifically mentioned this calculation almost certainly excludes human labour).

In such economies it is imperative that draught animals are able to work to their full potential when necessary, particularly for small-holder and subsistence farmers who rely on them for food production. Poor work output can delay cultivation tasks leading to reduced crop yields and consequently to lower overall farm productivity, particularly when growing seasons are short. Good working animals are often hired out to increase farm income once cultivation tasks at home are completed. If these cultivation tasks take longer than normal, the animals may not be available for hire to others, thus reducing the owner's income. A number of authors have stated that disease reduces the work output of draught animals on farms (Dharsana and Campbell, 1993; Lun *et al,* 1993; Partoutomo *et al,* 1985), however the evidence is largely anecdotal, with few experimental attempts to quantify the effects (Pearson, 1989b; Rukmana, 1979). Conversely work may make draught animals more susceptible to disease (Munzinger, 1982; Starkey, 1981). Hoffmann

and Dalgliesh (1985) suggested that sub-clinical infections in draught animals are of particular concern because they may go undiagnosed and consequently untreated for months even years.

Surra caused by *Trypanosoma evansi* is an important disease of large ruminants and horses throughout S.E. Asia, as has been indicated in section 2.3.3. In Indonesia the parasite has been identified on most of the islands in the archipelago where domestic livestock are reared (Dennig, 1976; Payne, 1989; Payne *et al*, 1991b). It is considered one of the most important livestock diseases in the country (Adiwinata and Dachlan, 1969; Dieleman, 1986; Winrock International Institute for Agricultural Development, 1986). Economic losses of up to US$ 28 million p.a. (Directorat Jenderal Peternakan, 1984) have been ascribed to *T. evansi* infection through mortality, reduced growth rates, infertility, abortions and reductions in draught animal power, however as Partoutomo, Ronohardjo, Wilson and Stevenson (1985) and Lun *et al* (1993) observed there is little quantitative data on these parameters. The only attempt to quantify the effects of *T. evansi* on work output has been by Rukmana (1979) who found that buffalo artificially infected with the disease ploughed 29% less land in one hour compared to uninfected animals. Winrock International Institute for Agricultural Development, (1986) estimated that surra significantly reduced the work output of 80% of the draught animals in some areas of Indonesia, although no evidence was given to support this figure.

In areas where *T. evansi* is endemic the disease in buffalo and cattle is usually chronic with low mortality rates, in horses it is acute and generally fatal unless treated (Bakkar, 1930; Payne, 1989). The disease causes few obvious symptoms in cattle and buffalo beyond chronic emaciation (Dieleman, 1986), however acute deterioration has been observed when food supplies drop or when animals are asked to perform hard work (Kraneveld and en Djaenoedin, 1948).

Stress has been implicated as a factor predisposing animals to the disease. As early as 1917 Doeve (1917) reported that adverse climatic conditions such as wind and rain increased the pathogenic effects of *T. evansi* in buffalo. He also observed that in Indonesia buffalo deaths due to surra were most common during the wet season planting of sugar cane when fodder was most scarce, leading him to suggest that variations in the responses of individuals to *T. evansi* infection were related to nutritional stress. More recently Wells, (1981) reported that in Vietnam, trypanosomosis is a problem in buffalo when they are moved from the mountains to the coast for work. Wells proposed that the stresses of walking and climatic changes might have increased the animals susceptibility to the disease although contact with new strains of the pathogen might also have been implicated. Work and in particular overwork has also been implicated in outbreaks of trypanosomosis in buffalo in Thailand (Löhr *et al*, 1985).

The aim of the present study was to quantify the impact of *T. evansi* infection on the work performance of swamp buffalo (***Bubalus bubalis***) in Indonesia. Two groups of buffalo were worked in pairs pulling weighted sledges round a track. One group was infected with *T. evansi* before being worked, the other group was stressed by work prior to infection. The animals were worked at a similar rate to buffalo on local farms.

The study was carried out at the Research Institute for Veterinary Science (RIVS), Bogor, West Java, Indonesia as part of a collaborative project on trypanosomosis between the ODA Animal Health Project Indonesia and RIVS. The author did all the work detailed below with some technical assistance from RIVS staff, with the exception of the parasitology which was carried out by staff of the RIVS parasitology department under the supervision of Richard Payne (ODA) and Ismu Prastyawati Sukanto (RIVS). Nutritional analyses of the feedstuffs used in the experiment were completed by the author at the CTVM in Scotland.

4.2 Materials & Methods

4.2.1 Animals

Twelve young uncastrated male buffalo (250 - 384 kg liveweight) were purchased locally for the study. At the time of purchase the bulls were parasitologically negative for *T. evansi* by the microhaematocrit centrifugation technique (MCHT) (Woo, 1970), nor did they have antibodies to *T. evansi* by ELISA using the technique described by Payne, Ward, Usman, Rusli, Djauhari and Husein, (1988).

The animals were paired for the duration of the experiment according to liveweight (LW), size and willingness to work together. The two lightest teams were allocated one to each of two groups at random (Table 4.1). The two medium and the two heaviest teams were allocated in the same way, giving three teams of two animals in each group. Teams were colour coded for ease of identification.

The six buffalo in group 1 were artificially infected with T. evansi and then worked in pairs for five weeks, 5 days/week pulling weighted sledges around a level track (work period 1, days 1 - 33 inclusive). Thereafter the animals were rested for two weeks, during which time they were treated with a trypanocide to eliminate the parasites before being worked for a further six weeks (work period 2, days 50 - 89). The six animals in group 2 were worked for five weeks before being infected with T. evansi. After infection they were rested for two weeks and then worked for a further six weeks. These animals were treated with trypanocide after work period 2. The experimental design is illustrated in Figure 4.1.

Before the experiment all animals were treated against ecto- and endoparasites with ivermectin (1% w/v) at a dosage of 1 ml/ 50 kg LW s.c. (Ivomec ®, Merck Sharp & Dohme Ltd., Hoddesdon, UK), against *Fasciola spp.* with triclabendazole

(10% w/v) at 12 mg/kg LW as oral drench (Fasinex ®, Ciba-Geigy, Cambridge, UK) and, as a precaution, with cymelarsan at 0.75 mg/kg LW i.m. (Mel-Cy ®, Rhone Merieux Ltd., Harlow, UK) against *T. evansi* in case they had become infected since purchase.

When they were not being worked the animals were housed in individual pens in a fly-proof animal house.

Group	Animal no.	Animal initial LW (kg)	Team	Team initial LW (kg)
1	402	298	White	594
1	405	296	White	
1	409	311	Red	633
1	410	322	Red	
1	400	326	Yellow	671
1	404	345	Yellow	
2	406	250	Green	518
2	408	268	Green	
2	403	306	Stripes	620
2	411	314	Stripes	
2	401	350	Blue	734
2	407	384	Blue	

Table 4.1 : Allocation of buffalo teams to groups by liveweight.

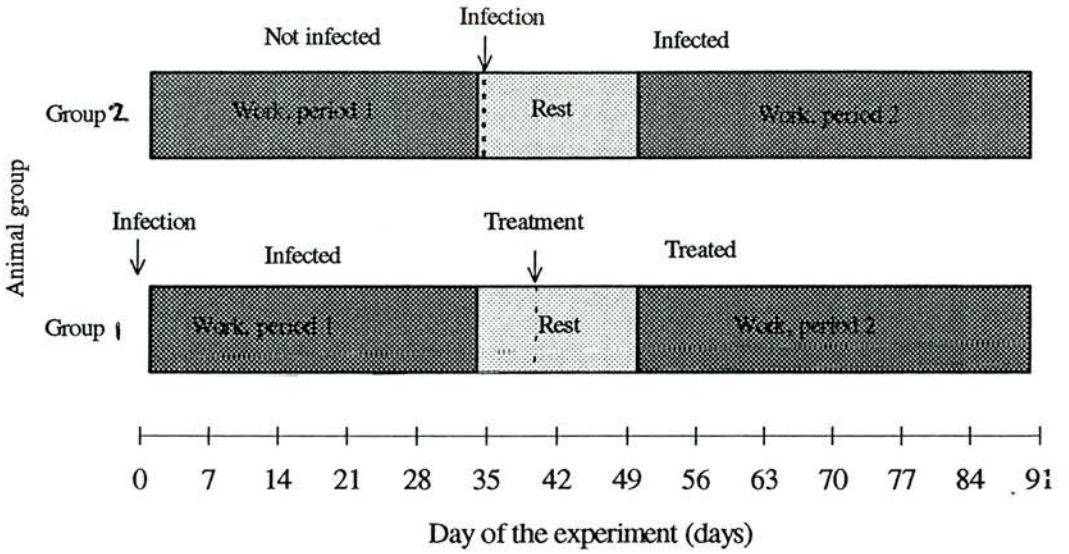


Figure 4.1 : The experimental design.

4.2.2 Animal nutrition

The buffalo were individually fed on fresh, chopped elephant grass (*Pennisetum purpureum*) supplemented with cattle concentrate feed. Each animal received approximately 1.7 times its metabolisable energy requirement for maintenance ($1.7 \times ME_{maint}$), calculated according to the AFRC (1993) recommendations for cattle using animal liveweights recorded prior to the experiments (day –38). Feeding recommendations for cattle were used because measurements of fasting metabolism in buffalo suggest that they are not dissimilar in their requirements to temperate cattle (Devendra, 1983). The amount of feed offered to each animal was recalculated prior to work period 2, to allow for any changes in animal LW which had occurred during period 1. Fresh weights of the respective feedstuffs offered to each animal are detailed in appendix 24.

On working days the buffalo received 1 kg of concentrate at 06.30 h before work and the balance of their ration at around midday after work. Water was

available *ad libitum* when they were not working. The daily food intake of each animal was monitored throughout each experiment. Samples of the feedstuffs offered were collected daily and bulked to provide a weekly sample for analysis.

Feed dry matter (DM) was determined by forced-air oven drying to constant weight at 55°C. Dried samples were ground to pass through a 1 mm screen before the gross energy, crude protein, acid detergent fibre, neutral detergent fibre and ash content were determined according to the Association of Analytical Chemists (1990).

4.2.3 Work

The buffalo were worked by being made to pull weighted sledges round a 100 m mud track using traditional shoulder yokes (Plate 4.1). The six teams were worked simultaneously for up to 2.5 h/day, 5 days a week, starting at 7.30 h each morning. Each team was encouraged to complete as many laps of the track as possible within the 2.5 h working day. Teams were retired early if either buffalo was judged by an independent observer to be showing one or more signs of severe fatigue or stress, as detailed below:

1. Very rapid, shallow breathing with tongue protruding and excessive salivation.
2. Staggering uncoordinated gait.
3. Repeated attempts to lie down or leave the track.
4. Repeated stopping and refusal to move.

During each working day the animals were rested for 20 minutes after 1.5 h work or sooner if they showed signs of fatigue. After rest the direction of travel was reversed, ensuring the two buffalo in each team performed similar amounts of work over the day. After work, the animals were hosed down with cold water to cool them.

Plate 4.1 : Two of the buffalo harnessed to their sledge with a shoulder yoke, reins and nose ropes are clearly visible.

Sledges were loaded with ballast sacks so that the total weight of each sledge plus driver was equal to 25% of the combined LW of the two buffalo pulling it. Drivers were allocated to specific teams for the duration of the studies barring accident or illness and rode on the sledges controlling the buffalo by means of reins attached to nose ropes. This is the normal method of controlling draught buffalo in Java. Ballast sacks were adjusted, prior to work period 2 to ensure that the load being pulled remained at 25% of team LW at the start of that period. Because of the adjustment of sledge weights two ADF test days (as detailed in section 3.1.2) were required in this study, one before each period to determine the ADF required by each team to pull its loaded sledge during each period.

Team lap times and the total number of laps completed by each team each day were recorded by an independent observer throughout the experiment. From these figures the daily distance travelled, average speed and total time worked were calculated for each team. In group 2 the distance travelled, work done, ADF, speed and elapsed work time of the blue team were recorded at minute intervals over each working day, using a load cell and odometer wired to a data logging device mounted on the sledge as detailed in section 3.1.1. The daily work done by each of the six teams was calculated from the corrected team ADF multiplied by the distance travelled by that team.

4.2.4 Energy expenditure for work

The daily net energy expenditure of each buffalo for work was estimated using the factorial method (Lawrence, 1985) detailed in section 3.1.3 with the following parameters:

L = load carried (kg) = weight of the yoke, 7.5 kg

A = energy used by swamp buffalo to move 1 kg of body weight 1 m horizontally, 2.09 J/m (Lawrence and Stibbards, 1990)

B = energy used by swamp buffalo to move 1 kg of applied load 1 m horizontally, 2.6 J/m (Agricultural Research Council, 1980; Lawrence and Stibbards, 1990)

C = efficiency of swamp buffalo doing mechanical work, 0.37
(Lawrence and Stibbards, 1990)

4.2.5 Track condition

The track surface condition was assessed prior to work each day on an arbitrary scale of 1 to 5 with additional plus marks to indicate when the mud was particularly deep and sticky/ slippery (Table 4.2). Scoring was done by the same person throughout.

Score	Track condition
1	Significant surface water, very wet and slippery
2	Wet and slippery
3	Sticky mud
4	Drying mud
5	Dry track
+	Sticky / slippery deep mud
++	Sticky / slippery very deep mud (over boot height in places)

Table 4.2 : Scoring system used for assessing track surface condition.

4.2.6 Animal liveweights

The buffalo were weighed once a week to monitor liveweight changes, using an electronic balance.

4.2.7 Animal body temperatures

The rectal temperature of each buffalo was measured daily at 06.30 h in the animal house throughout the experiment using a clinical mercury thermometer, starting two weeks before the first work period in order to establish baseline values for each animal.

The temperature of each animal was also monitored at regular intervals during work each day, using rectal probes comprising platinum resistance thermometers encased in stainless steel tubes. The probes once inserted were held in place by cruppers round the animals' tails attached to girth straps. They appeared to cause no discomfort to the animals and were easily reinserted if they came out when the animals defaecated. Blue team temperatures were recorded automatically once per minute by the data logger unit which was connected to the rectal probes via cables running up the draw chain and along the yoke to the two animals. The temperatures of the other 10 animals were recorded manually once every 15 minutes from battery powered liquid crystal display (LCD) units connected to the two rectal probes. The LCD units were mounted on the girth strap of one animal in each team.

For analysis temperature changes during the course of the working day were split into three time intervals; (i) the temperature rise between commencing work and the mid-morning 15 minute rest interval; (ii) the change during the rest interval; (iii) and the rise thereafter until the end of work that day.

4.2.8 Infection details

Trypanosome populations for the infections were initially expanded in outbred laboratory mice from cryogenically preserved populations. All aspects of infection and treatment were carried out by staff of the Parasitology Department of RIVS.

Group 1

Two days before work started the animals were infected with an Indonesian stock of *T. evansi* (Bakit 362), each animal receiving 2×10^7 trypanosomes by i.v. injection. Animals 402, 405 and 410 were given second injections of 2×10^8 parasites of the same strain 10 days later (day 8). At the end of work period 1 all animals were

treated with cymelarsan at a dose rate of 0.75 mg/kg LW i.m. to eliminate the trypanosomes; buffalo 409 was treated on day 34, the others on day 40.

Group 2

The six buffalo in group 2 were infected with a different Indonesian stock of *T. evansi* (Garut 197) immediately after a five week work period (day 34). Each animal received a single dose of 2×10^7 trypanosomes, by the same route as that used for group 1. These animals were treated with cymelarsan (0.75 mg/kg LW i.m.) on day 90 after work period 2.

4.2.9 Parasitology & haematology

Blood samples were collected daily from each animal by venepuncture of a marginal ear vein into heparinised microhaematocrit capillary tubes for assessment of PCV and as appropriate parasitaemia. Sampling started 18 days before work period 1. Once the buffalo started work the frequency of collection was increased to twice a day on working days, immediately before and after work, nominally 06.30 h and 11.00 h. On non-working days a single sample was collected at 06.30 h.

PCV was determined using a proprietary reader (Hawksley & Sons, Lancing, UK) after microhaematocrit centrifugation of the capillary tubes. Parasites were detected by examining the plasma-leukocyte interface of each tube in situ, according to the microhaematocrit centrifugation technique (MHCT) (Woo, 1970). In this experiment animals which were parasitaemic by MHCT were considered to be infected, animals in which no parasites could detected by MHCT were considered to be uninfected. The parasitaemias of any trypanosome positive samples were estimated by the dark ground illumination (DG) method of Murray, Murray and McIntyre (1977) and expressed as log equivalent values (LEV) (Walker, 1969) of the

average number of parasites per 20 microscope fields at a magnification of ×400 (Appendix 9). For statistical analysis parasitaemias in excess of 100 trypanosomes per field were given a nominal LEV of 5, samples which were MHCT positive but had less than 1 trypanosome per 20 fields on DG examination were given an LEV of 0.25.

4.2.10 Meteorology

The wind speed, black bulb temperatures and relative humidity were recorded at the track site at hourly intervals when the buffalo were working. Maximum and minimum temperatures and relative humidity in the animal house were recorded daily at 11.00 h.

4.2.11 Data analysis

Data from the two groups of animals was analysed separately, with cross comparisons made as appropriate. In the event of any animal failing to become infected with *T. evansi* as intended, group comparisons were made without the data from that individual or team, where a single animal within a team was infected team parameters were compared with and without the data for that team. Unless otherwise stated "period" is synonymous with "work period", period differences referring to differences between the work periods 1 and 2. All percentage data was transformed before analysis using a standard angular transformation (section 3.3).

Differences in the parasitaemia profiles of the buffalo in the two groups over the first 32 days after infection, were tested using a series of Mann-Whitney tests. Within each group paired Wilcoxon tests were used to identify any significant changes in parasitaemia and PCV over the working day, with the data from each buffalo being tested separately. Mann-Whitney tests were also used to analyse period differences in; (i) the ADF required by the blue team to pull its sledge; (ii) working speeds of

individual buffalo teams; (iii) animal body temperatures at 06.30 h each morning; (iv) rates of body temperature change of individuals over the three time intervals of the working day (as defined in section 4.2.7); and (v) air temperatures within the buffalo house. Differences in team speeds in each work period were compared with Kruskal-Wallis tests.

The rate of liveweight change of each buffalo during each period was calculated from weekly weights by the least squares method of linear regression, the gradient of the line of best fit was taken as the rate of liveweight change. The same regression technique was used to examine the relationships between; (i) the time a team started work in the morning and the subsequent length of the working day; (ii) start time and working speed; (iii) the LEV parasitaemia of animals in a team on a particular day and the time worked by the team on that day; (iv) LEV parasitaemia and working speed; and (v) LEV parasitaemia and 06.30 h body temperature. Analysis of variance (ANOVA) was used to determine whether any correlations seen were linear.

Within each group, period differences in the percentages of days when PCV's at 06.30 h were below specified threshold levels were compared using paired T-tests to analyse transformed data. The threshold levels used were; (i) days when an animal's PCV fell below the lower 95% confidence limit (CL) of its pre-experiment mean PCV nominally "anaemia days"; and (ii) days when an animal's PCV was less than 25%. Group differences in these two parameters and in pre-experiment mean PCV's were analysed using unpaired T-tests. Differences in resting animal body temperatures during periods 1 and 2 were similarly tested, by comparison of the percentage of days in each period when an animal's body temperature at 06.30 h exceeded the upper 95% CL of its pre-experiment mean.

Paired T-tests were also used to analyse period differences in; (i) rates of animal liveweight change; (ii) elephant grass intakes expressed as a percentage of feed offered; (iii) gross energy intake per unit of metabolic liveweight ($LW^{0.75}$); and crude

protein intake /$LW^{0.75}$. Group differences in these three data sets within each period were tested for significance using unpaired T-tests as were group differences in animal liveweights at the start and finish of the experiment.

Period differences in the composition of the feeds offered to the animals were analysed using T-tests, as were black bulb temperatures and relative humidities measured at the trackside while the animals were working. The feed composition data with the exception of the gross energy results, was transformed before being tested (section 3.3). T-tests were also used to compare period differences in (i) time worked each day;(ii) distance travelled; (iii) work output per unit of metabolic LW; and (iv) power output per unit of metabolic LW, for individual teams; together with (v) net energy expenditures of individual animals for work (NE_{work}) per unit of metabolic LW. Within each period the significance of team differences in; (i) time worked; (ii) distance travelled; and (iii) speed were tested by ANOVA. Period variations in daily total team work output and power output were not analysed because of the different sledge loads used in period 2.

4.3 Results

4.3.1 Trypanosome infections

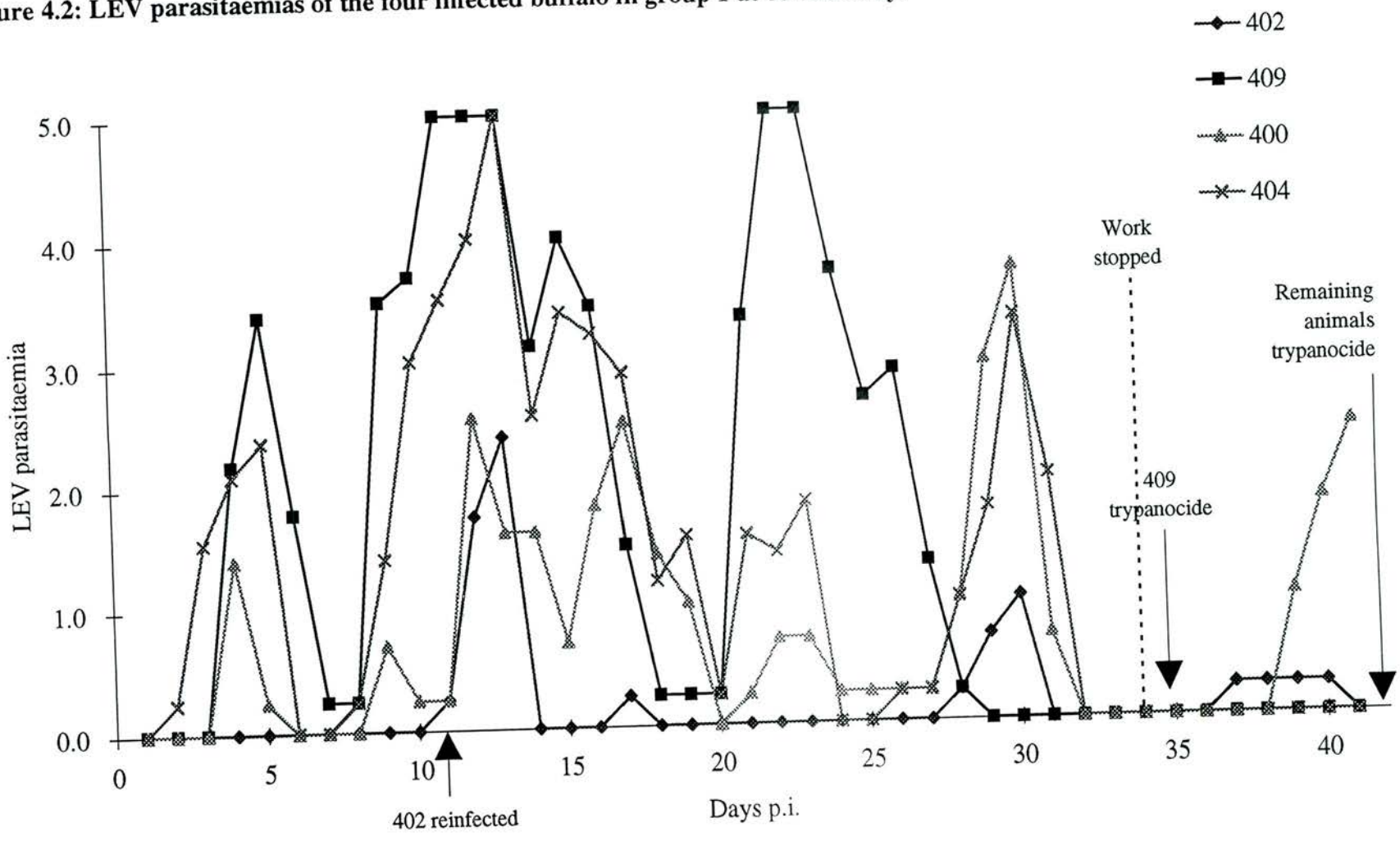
Group 1

Only four of the six buffalo infected with *T. evansi* (Bakit 262) developed patent parasitaemias during the five week period, despite three animals being rechallenged. One animal in the white team, number 405 and one in the red team, no. 410 did not become infected. Most of the time parasitaemias in the four infected animals fluctuated between 1 trypanosome/20 fields and 20 trypanosomes/field. All four animals experienced several distinct parasitaemic peaks, however the magnitude of the peaks varied considerably between individuals as illustrated in Figure 4.2. Buffalo 402 never developed a parasitaemia above 10 trypanosomes/field by DG, whereas no. 409 suffered two parasitaemic peaks of >100 trypanosomes/field. The parasitaemia profiles of individual animals are summarised in Table 4.3.

Work had no short-term effect on the parasitaemias of any of the trypanosome positive buffalo in that parasitaemias immediately before and after work each day were not significantly different, by paired Wilcoxon tests (P for the four animals ranged from 0.2 to 0.8, n = 25, with the exception of buffalo 402 when n = 19). Daily parasitaemia data for each animal is given in appendix 10.

Thirty days after infection (day 29 of the experiment) buffalo 409 (Red) was diagnosed by the parasitology dept. of RIVS as having a concurrent *Anaplasma marginale* infection. All buffalo in both groups were immediately treated with imidocarb dipropionate (12% w/v) at 2.5 ml/100 kg s.c. (Imizol, Pitman-Moore Ltd., Crewe, UK), buffalo 409 was also given oxytetracycline hydrochloride (20 mg/kg i.v.,

Figure 4.2: LEV parasitaemias of the four infected buffalo in group 1 at 06.30h daily.



Buffalo no.	Team	Days prepatent	No. days sampled once MHCT +ve	Days MHCT +ve	Days tryps. > 1/20 fields+	Days tryps. > 1 /field+	Days tryps.> 10 /field+	Days tryps. > 20 /field+	Days tryps. > 50 /field+	Days tryps. > 100 /field+
402	White	2	31	11	4	1	0	0	0	0
			%	35.5	12.9	3.2	0.0	0.0	0.0	0.0
405	White	Did not become infected at any time								
409	Red	4	31	25	19	16	13	12	8	5
			%	80.6	61.3	51.6	41.9	38.7	25.8	16.1
410	Red	Did not become infected at any time								
400	Yellow	4	38	27	19	5	1	1	1	0
			%	71.1	50.0	13.2	2.6	2.6	2.6	0.0
404	Yellow	2	40	26	21	11	7	4	2	1
			%	65.0	52.5	27.5	17.5	10.0	5.0	2.5

+ By the DG technique examining 20 microscope fields.

Table 4.3 : Parasitaemia profiles of the buffalo in group 1.

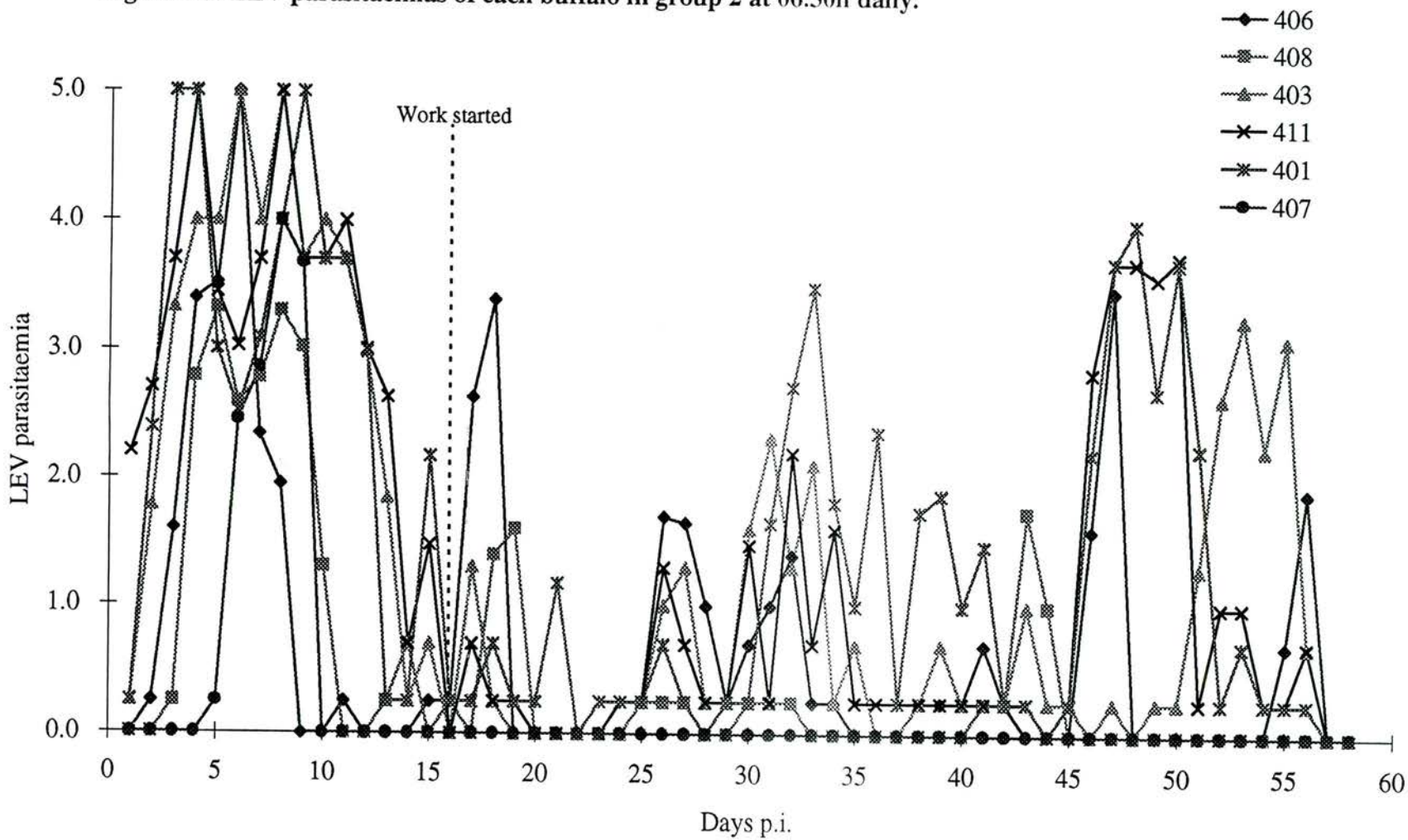
Terramycin LA, Pfizer, Sandwich, UK). Despite this the infection recurred on day 72. The animal was treated as before with imizol and terramycin. On day 47, buffalo 400 (Yellow) received a second injection of imizol as it too was suspected of having anaplasmosis by the RIVS parasitology department.

Group 2

All the buffalo became parasitaemic after infection with *T. evansi* (Garut 197), with high parasitaemias recorded within a week of infection. One animal 407 (Blue) appeared to rapidly self-cure, becoming MHCT negative from 10 days p.i.. Parasitaemias in the other five animals remained high for the first fortnight after infection, but declined thereafter with small sporadic peaks until 45 days p.i. (day 79 of the experiment) when the parasitaemias of three animals, one in each team, increased markedly (Figure 4.3). The parasitaemia of a fourth buffalo no. 403 rose five days later. Individual parasitaemia profiles are given in Table 4.4.

Work caused small but significant short term increases in the parasitaemias of two animals in this group, 406 and 411 by Wilcoxon tests (C = 38.5, P = 0.04 and C = 47.5, P = 0.01, respectively, n = 30). The parasitaemia of buffalo 406 (Green) rose from an average of 1 trypanosome/20 fields before work each day to 1 trypanosome/14 fields after work, and that of 411 (Stripes) rose from 1 trypanosome/15 fields to 1 trypanosome/9 fields. Work had no significant short term effect on the parasitaemias of the other four animals in the group (P ranged from 0.2 to 1.0, n = 30). The daily parasitaemias of each animal are given in appendix 11.

Figure 4.3: LEV parasitaemias of each buffalo in group 2 at 06.30h daily.



Buffalo no.	Team	Days prepatent	No. of days sampled once MHCT +ve	Days MHCT +ve	Days tryps. > 1/20 fields+	Days tryps. > 1 /field+	Days tryps.> 10 /field+	Days tryps. > 20 /field+	Days tryps. > 50 /field+	Days tryps. > 100 /field+
406	Green	2	57	31	19	7	5	5	1	1
			%	54.4	33.3	12.3	8.8	8.8	1.8	1.8
408	Green	3	56	23	12	6	3	1	0	0
			%	41.1	21.4	10.7	5.4	1.8	0.0	0.0
403	Stripes	1	58	45	29	16	11	9	8	2
			%	77.6	50.0	27.6	19.0	15.5	13.8	3.4
411	Stripes	1	58	50	30	19	14	12	10	2
			%	86.2	51.7	32.8	24.1	20.7	17.2	3.4
401	Blue	1	58	53	32	21	12	10	9	3
			%	91.4	55.2	36.2	20.7	17.2	15.5	5.2
407	Blue	5	54	5	4	4	2	2	1	0
			%	9.3	7.4	7.4	3.7	3.7	1.9	0.0

+ By the DG technique examining 20 microscope fields.

Table 4.4 : Parasitaemia profiles of the buffalo in group 2.

A comparison of parasitaemia profiles in groups 1 & 2.

Parasitaemias in group 2 appeared to increase much more rapidly after infection and remained consistently higher, than those in group 1 (Figure 4.3 compared to Figure 4.2). However none of the parasitaemia parameters used revealed any significant differences between the two infections over the first 32 days p.i. (Table 4.5).

Parameter	Group 1 (n = 4)		Group 2 (n = 6)		MW test
	median	s.i.r.	median	s.i.r.	P_{group}
Days to patency	3.0	1.0	1.5	1.25	0.32 ns
Days MHCT +ve	24.5	5.75	22.5	6.13	1.00 ns
Days DG +ve	17.5	6.75	15.5	5.38	0.75 ns
Days > 1 tryp./field	7.5	6.50	8.5	3.88	0.75 ns
Days > 10 tryps./field	4.0	5.63	6.0	3.25	0.59 ns
Days > 20 tryps./field	2.5	4.88	5.0	3.25	0.59 ns
Days > 50 tryps./field	1.5	3.13	3.5	3.25	0.92 ns
Days > 100 tryps./field	0.5	2.00	1.5	1.13	0.83 ns

Table 4.5 : Median parasitaemia profiles for each group from blood samples collected at 06.30 h over the first 32 days p.i. with Mann-Whitney tests for significance of differences.

4.3.2 Blood packed cell volume

Group 1

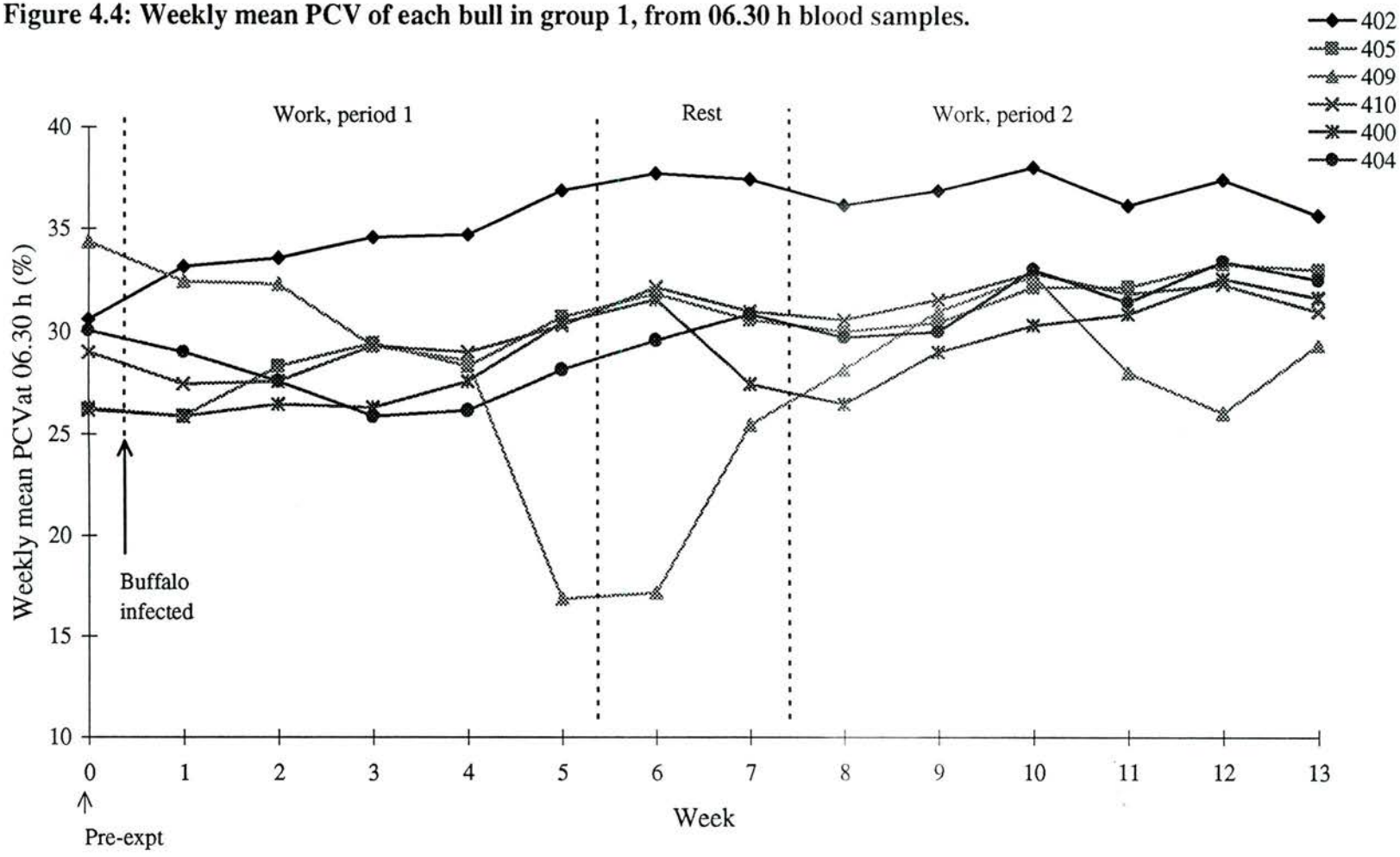
The 06.30 h weekly mean PCV's of two of the four infected animals, buffalo 400 and 404, remained almost static over work period 1, increasing slightly in week 5 (Figure 4.4) whereas that of buffalo 402 which was also infected, rose consistently over the period. The PCV of buffalo 409 which developed anaplasmosis whilst already infected with trypanosomosis fell each week from the start of the trial,

declining markedly from week 4 when its PCV fell from 32% to just 14% in 10 days. The PCV of one of the uninfected animals increased over period 1, that of the other animal remained static until week 5 when it increased slightly. In work period 2 once the animals had been treated, all PCV's rose steadily with the exception of the PCV of animal 409, which fell again after week 10, following a recurrence of the anaplasmosis.

The proportion of anaemia days (as defined in section 4.2.11) experienced by the four infected buffalo did not differ significantly by paired T-test, in period 1 when the animals were parasitaemic compared to period 2 when they had been treated ($T = 1.72$, $P = 0.18$, $n = 4$). Similarly there was no significant difference in the proportion of days when the 06.30 h PCV's of these four animals were less than 25% in periods 1 and 2, by paired T-test ($T = 2.80$, $P = 0.07$, $n = 4$). Excluding the data from buffalo 409 which had anaplasmosis did not affect the significance of either result. The number of anaemia days and days when PCV was below 25% experienced by each animal during the experiment are detailed in Table 4.6.

The impact of work on PCV over the working day differed between animals. Work caused a significant decline in the PCV's of 402 and 409 between 06.30 h and 11.00 h each day, by paired Wilcoxon tests ($C = 1158$, $P < 0.001$ and $C = 775$, $P = 0.001$ respectively, $n = 53$) whereas it resulted in increases in the PCV's of 400 and 404 ($C = 33$ and 136 respectively, $P < 0.001$ for both animals, $n = 51$). The PCV's of 405 and 410, the two animals that did not become infected, did not change significantly over the working day ($C = 352$, $P = 0.54$ and $C = 512$, $P = 0.30$ respectively, $n = 53$).

Figure 4.4: Weekly mean PCV of each bull in group 1, from 06.30 h blood samples.



Buffalo no.	Pre-expt. mean PCV (%) (n = 17)	Lower 95% CL+ of pre-expt. mean (%)	Period 1 (infected)			Period 2 (treated)		
			No. of days examined	No. of anaemia days (PCV < CL^+)	No. of days PCV < 25%	No. of days examined	No. of anaemia days (PCV < CL^+)	No. of days PCV < 25%
402	30.6	28.78	33	1	0	40	0	0
			%	3.0	0.0	%	0.0	0.0
405[$]	26.2	24.17	33	2	2	40	0	0
			%	6.1	6.1	%	0.0	0.0
409	34.4	31.74	33	22	7	40	27	1
			%	66.7	21.2	%	67.5	2.5
410[$]	29.0	27.02	33	8	1	40	1	0
			%	24.2	3.0	%	2.5	0.0
400	26.2	24.46	33	7	7	40	3	3
			%	21.2	21.2	%	7.5	7.5
404	30.1	28.15	33	25	2	40	4	0
			%	75.8	6.1	%	10.0	0.0

CL^+ 95% confidence limit of pre-experiment mean

[$] animal did not become parasitaemic

Table 4.6 : 06.30 h PCV parameters for each buffalo in group 1 over the two working periods.

Group 2

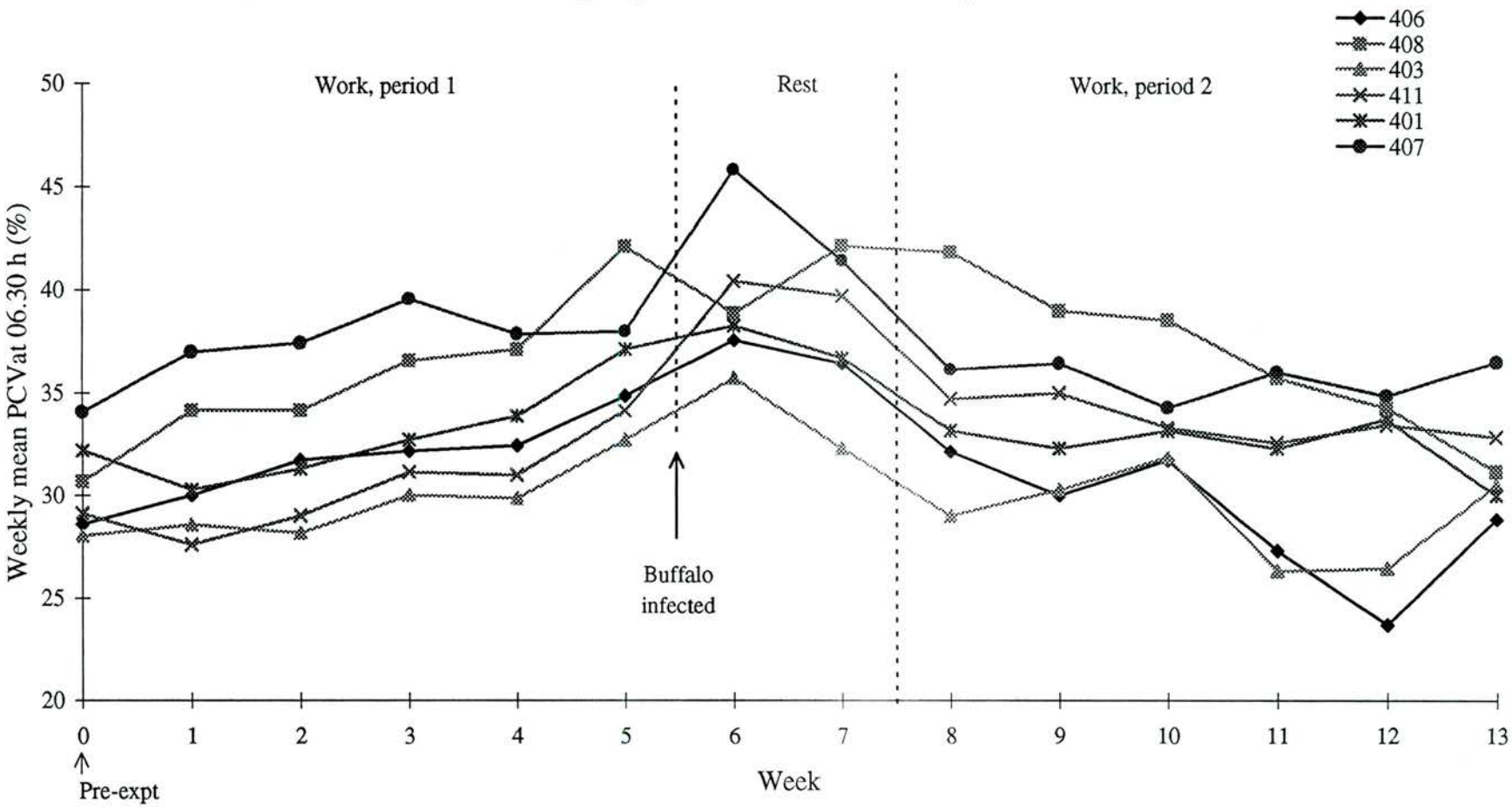
The 06.30 h PCV's of all the animals in this group increased over work period 1, but fell once the animals were infected with *T. evansi* (Figure 4.5). Neither the proportion of anaemia days nor the proportion of days when the buffalo PCV's were below 25%, differed significantly in periods 1 and 2, by paired T-test ($T = 1.09$, $P = 0.33$ and $T = 0.93$, $P = 0.39$ respectively, $n = 6$). Counting period 2 anaemia days and days with PCV below 25% from the point of infection (day 34), rather than from the day when work started (day 50) had no effect on the significance of these results. The number of anaemia days and days with PCV less than 25% at 06.30 h for each animal in each period are given in Table 4.7.

The PCV's of buffalo 406, 408 and 407 fell very significantly over the working day, by paired Wilcoxon tests ($C = 917$, 1262 and 980 respectively, $P < 0.001$, $n = 53$ for all three animals). In contrast the PCV of animal 401 increased very significantly between 06.30 h and 11.00 h ($C = 157$, $P < 0.001$, $n = 53$). The PCV's of 403 and 411 did not change ($C = 233$, $P = 0.27$ and $C = 420$, $P = 0.90$ respectively, $n = 53$).

A comparison of PCV parameters in groups 1 & 2.

In work period 1, group 1 animals which were infected suffered from a significantly higher percentage of anaemia days and days when PCV < 25% than group 2 which were uninfected at that time (Table 4.8). If however the results for buffalo 409 are excluded from the group 1 data, group differences in these two parameters are no longer significant, by T-test (anaemia days, $T = 1.73$, $P = 0.13$; PCV < 25%, $T = 2.13$, $P = 0.07$; $n_{1,2} = 3,6$). In period 2 there were no significant group differences in either percentage of anaemia days, or days when PCV < 25% (Table 4.8). Excluding the data from animal 409 did not alter the significance of the

Figure 4.5: Weekly mean PCV of each bull in group 2, from 06.30 h blood samples.



Buffalo no.	Pre-expt. mean PCV (%) (n = 17)	Lower 95% CL^+ of pre-expt. mean (%)	Period 1 (not infected)			Period 2 (infected)		
			No. of days examined	No. of anaemia days (PCV < CL^+)	No. of days PCV < 25	No. of days examined	No. of anaemia days (PCV < CL^+)	No. of days PCV < 25
406	28.6	26.40	33	0	0	40	8	8
			%	0.0	0.0	%	20.0	20.0
408	30.7	28.06	33	0	0	40	1	0
			%	0.0	0.0	%	2.5	0.0
403	28.0	26.92	33	0	0	40	10	2
			%	0.0	0.0	%	25.0	5.0
411	29.1	27.45	33	5	0	40	0	0
			%	15.2	0.0	%	0.0	0.0
401	32.2	29.72	33	9	1	40	9	0
			%	27.3	3.0	%	22.5	0.0
407	34.1	31.84	33	1	0	40	6	0
			%	3.0	0.0	%	15.0	0.0

CL^+ 95% confidence limit of pre-experiment mean

Table 4.7 : 06.30 h PCV parameters for each buffalo in group 2 over the two working periods.

PCV parameter	Group 1			Group 2			T-test	
	mean	s.e.	n	mean	s.e.	n	T	P_{group}
Pre-experiment PCV (%)	29.4	1.25	6	30.4	0.95	6	0.67	0.52 ns
Percentage of anaemia days in period 1	41.7	17.60	4	7.6	4.61	6	2.36	0.046 *
Percentage of anaemia days in period 2	21.2	15.60	4	14.2	4.31	6	0.26	0.80 ns
Percentage of days PCV<25 in period 1	12.1	5.39	4	0.5	0.51	6	2.80	0.02 *
Percentage of days PCV<25 in period 2	2.5	1.77	4	4.2	3.27	6	0.05	0.96 ns

(Group 1 means are calculated from the four infected animals, with the exception of the pre-experiment mean PCV.)

Table 4.8 : Mean PCV parameters for groups 1 and 2, with statistical analysis of differences using transformed data.

period 2 results. The mean pre-experiment PCV's of the buffalo in the two groups were almost identical.

There were no significant differences in the proportions of anaemia days nor in days when PCV < 25% in groups 1 and 2 when both groups were infected, by T-test (T = 1.67, P = 0.13 and T = 1.40, P = 0.20, respectively, $n_{1,2}$ = 4,6). Similarly there were no significant differences in either of these parameters when comparing the results for group 1 once treated with the results for group 2 when the animals were uninfected (anaemia days, T = 1.01, P = 0.34; PCV < 25%, T = 1.24, P = 0.25; $n_{1,2}$ = 4,6). Excluding the data for buffalo 409 did not alter the significance of any of these results.

4.3.3 Monitoring average draught force to calculate work output

The ADF required by the blue team to pull its sledge fluctuated markedly from day to day during the experiment as track surface conditions changed (Figure 4.6), ranging from 8 to 14.8% of team LW. On days when the surface was very wet and slippery (track scores 1 to 2) the ADF required by the animals was less than when the track was covered in sticky or drying mud (scores 3 to 4) as illustrated in Figure 4.7. The ADF as a percentage of team LW, required by the blue team to pull their sledge in period 2, was significantly higher than that required in period 1, by Mann Whitney test (W = 414.5, P = 0.03, $n_{1,2}$ = 21, 28). The period 2 median was 11.7% s.i.r. 1.14% compared to a median of 10.9% s.i.r. 1.36% in period 1. Daily ADF measurements for the blue team are given in appendix 12.

Changes in track conditions on the first ADF test day (section 3.1.2) prior to period 1 resulted in increases in ADF over time (Table 4.9). ADF increased as the day progresssed because of the changing track conditions. The tests were

Figure 4.6 : ADF required by the blue team to pull its sledge on each work day.

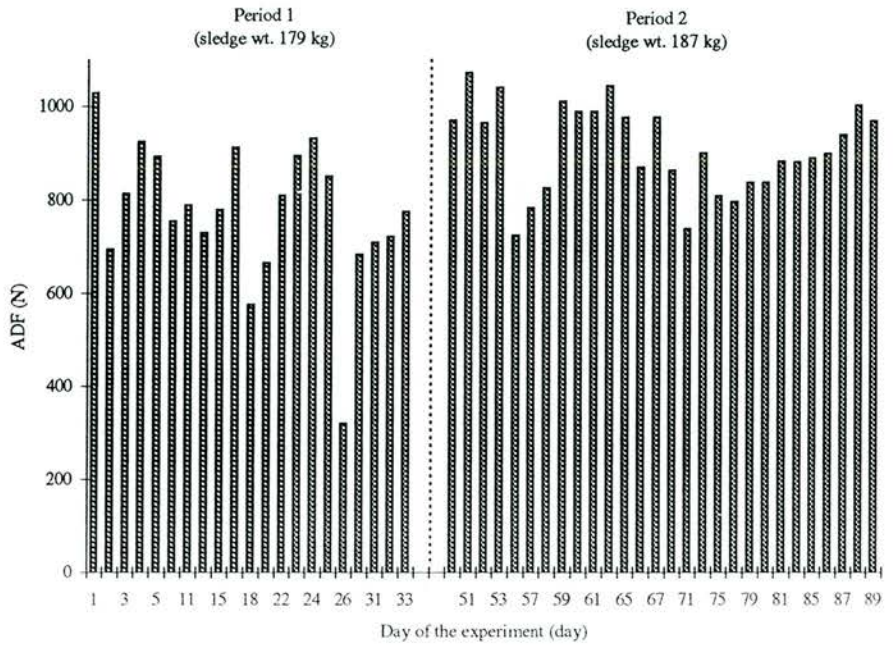
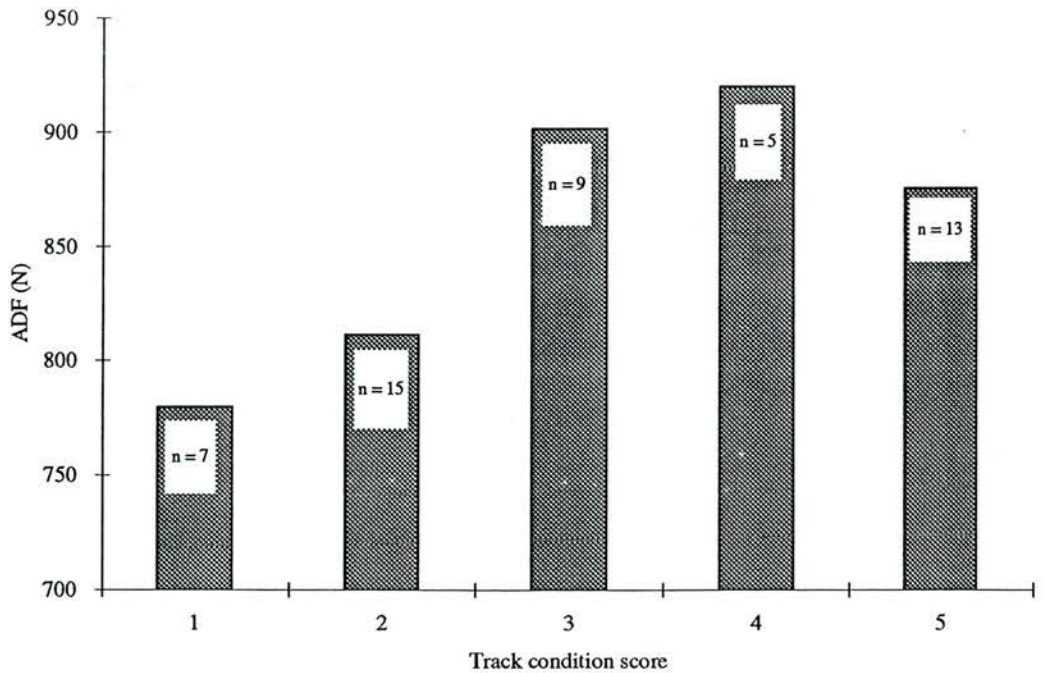


Figure 4.7: Mean ADF of the blue team over the experiment against track condition score.



ADF TEST DAY 1 (day –13)				ADF TEST DAY 2 (day –12)			
Track score	**Team**	**ADF (% LW)**		**Track score**	**Team**	**ADF (% LW)**	
		mean ± s.e.	**n**			**mean ± s.e.**	**n**
2	Blue	10.1 ± 0.13	10	2+	White	10.1 ± 0.23	13
(wet & slippery)	Red	13.3 ± 0.17	14	2+	Yellow	10.8 ± 0.26	8
	Stripes	12.6 ± 0.18	12	2+	Green	10.8 ± 0.16	8
	Green	16.0 ± 0.24	13	2+	Stripes	9.7 ± 0.12	9
(sticky)	Yellow	15.9 ± 0.16	5	2+	Red	11.5 ± 0.23	9
3+	White	16.4 ± 0.16	7	2+	Blue	9.5 ± 0.16	12

(Results recorded in the order in which the data was collected. On the first day ADF increased as the day progressed because of the changing track conditions. The loads pulled by the teams were the same on both days.)

Table 4.9 : ADF measurements on two consecutive test days prior to period 1, expressed as percentages of team LW on day –15.

therefore repeated the following day when track conditions remained constant whilst all 6 teams from the two groups were tested. The data obtained on this second test day was then used to calculate ADF correction factors for period 1. Another ADF test day was held prior to period 2, to derive new correction factors after sledge weights had been adjusted to 25% of team LW at the start of that period. The ADF test results for each team prior to each period are given in appendix 13.

4.3.4 Work performance

All six teams completed the two work periods, although there was a sharp decline in the work output of the three infected group 2 teams towards the end of period 2, some 45 days after infection. There was a similar fall in the work output of the treated group 1 teams at that time. The length of the working day varied markedly throughout the study from 20 minutes on a day when rain forced work to be abandoned, to almost three hours on cool overcast days. There were noticeable differences between teams in their willingness to work, irrespective of infection status, some buffalo appeared temperamentally better suited to work than others. Animal 411 (stripes) showed signs of chronic respiratory distress throughout the experiment with laboured noisy breathing and coughing. This was possibly indicative of a previous respiratory tract infection, however nothing was found on veterinary examination and the animal was pronounced fit to continue working.

Work output

Work outputs per unit of metabolic liveweight ($LW^{0.75}$) of all six teams rose over the first week of each period as the animals became accustomed to the work demanded of them. Thereafter they fluctuated from day to day reflecting changes in the many variables known to influence work output, before declining towards the end of period 2 (Figure 4.8 and Figure 4.9). The work outputs $/LW^{0.75}$ of all six teams on any one day were similar. On day 26 for example, the track was very greasy on top with a hard pan beneath (track score 2+ on top, 5 below), consequently the ADF required by the blue team to pull their sledge fell to 321 N (Figure 4.6), causing the marked fall in the work outputs $/LW^{0.75}$ of all six teams seen in Figure 4.8 and Figure 4.9.

There were no significant period differences in the work outputs $/LW^{0.75}$ of any of the six teams (Table 4.10). Mean total work outputs for each team in each period are presented in Table 4.10 in addition to work outputs $/LW^{0.75}$ merely for comparison with other studies. Weekly mean work outputs for each team in each period of the experiment are given in appendix 14.

Within period 1 there was no significant difference in the mean work output $/LW^{0.75}$ of the infected yellow team and the three uninfected group 2 teams by ANOVA ($F = 0.60$, $P = 0.52$, $df = 1,2$). Similarly in period 2 the mean work output $/LW^{0.75}$ of the treated yellow team was not significantly different to the mean output of the infected group 2 teams ($F = 0.74$, $P = 0.48$, $df = 1,2$). Results for the two group 1 teams in which only one animal became infected with *T. evansi*, namely the white and red teams, were excluded from these analyses to allow a straight comparison of infected and uninfected teams within each period. There was also no significant group difference in the mean work output $/LW^{0.75}$ of the yellow team compared to those of the three group 2 teams when all four teams were infected

Figure 4.8 : Daily work output per unit of metabolic LW of each team in group 1.

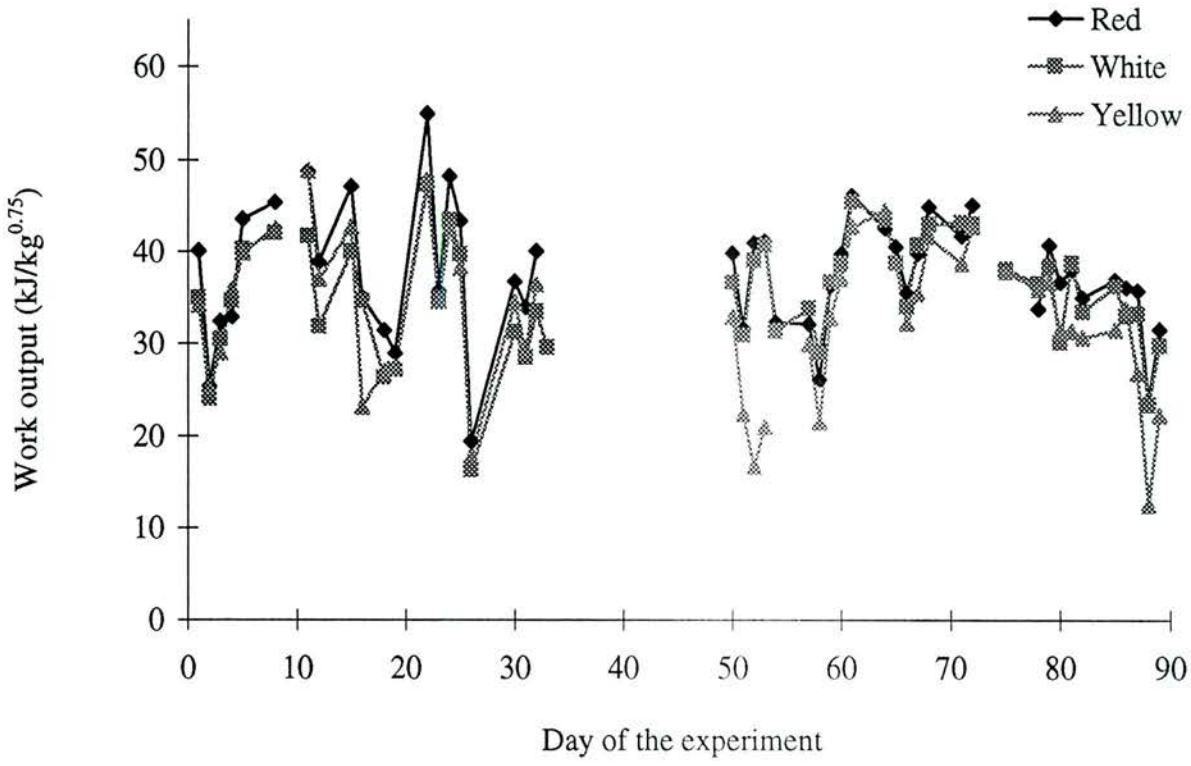
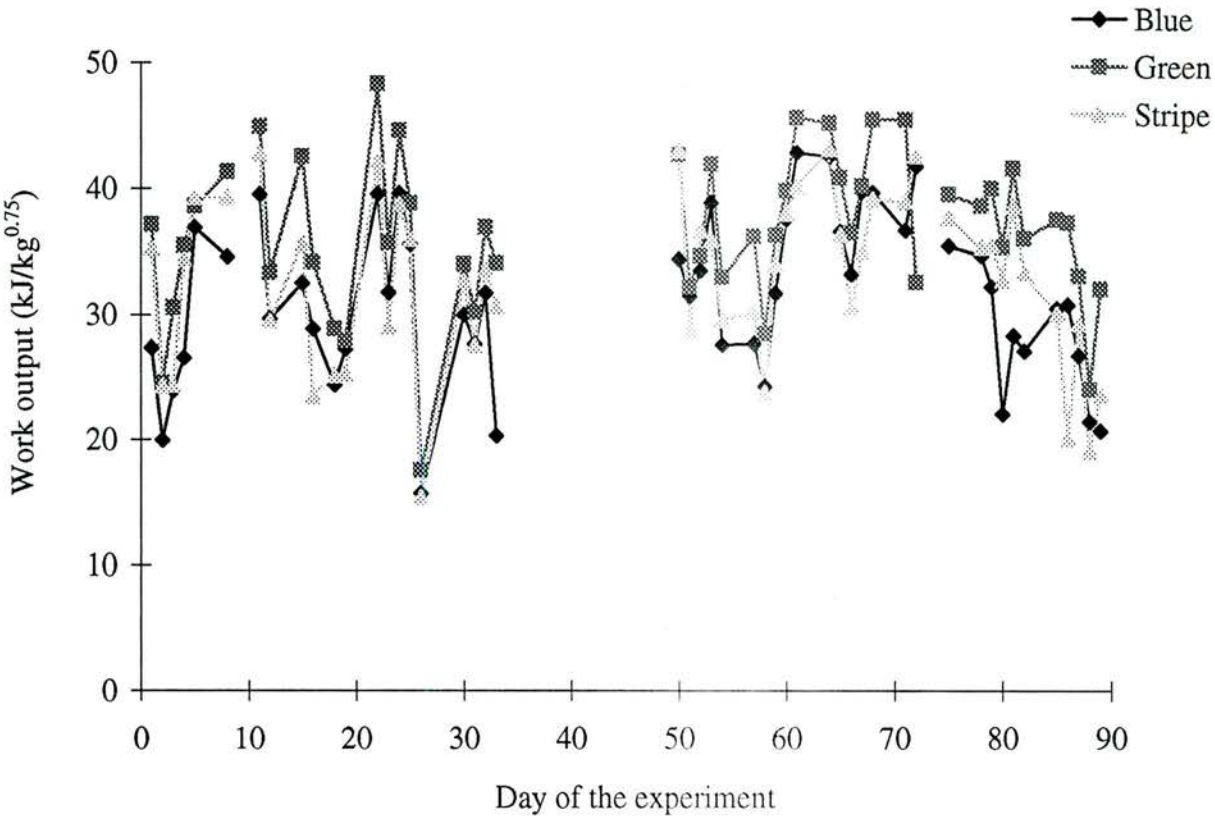


Figure 4.9: Daily work output per unit of metabolic LW of each team in group 2.



($F < 0.00$, $P = 0.98$, $df = 1,2$), and no significant group difference when yellow was treated and the group 2 teams were uninfected ($F = 0.01$, $P = 0.94$, $df = 1,2$). Including the data from the white and red teams in the group 1 results did not alter the significance of any of these analyses.

Team	Work output /$LW^{0.75}$ ($kJ/kg^{0.75}$) Period 1			Period 2			T-tests	
	mean	s.e.	n	mean	s.e.	n	T	P_{period}
Group 1	**Infected**			**Treated**				
White	33.9	1.62	21	36.5	0.99	28	1.39	0.17 ns
Red	38.1	1.95	20	37.2	1.05	27	0.40	0.69 ns
Yellow	34.7	1.85	20	31.9	1.59	27	1.14	0.26 ns
Group 2	**Uninfected**			**Infected**				
Green	35.2	1.58	21	37.6	1.01	28	1.35	0.18 ns
Stripes	31.7	1.55	21	33.6	1.23	28	1.02	0.31 ns
Blue	29.7	1.45	21	32.5	1.21	28	1.51	0.14 ns
Total work output per team (MJ/day)								
Group 1	**Infected**			**Treated**				
White	4.13	0.196	21	4.81	0.132	28		
Red	4.76	0.245	20	4.85	0.137	27		
Yellow	4.53	0.241	20	4.45	0.222	27		
Group 2	**Uninfected**			**Infected**				
Green	3.90	0.177	21	4.56	0.121	28		
Stripes	3.89	0.191	21	4.45	0.163	28		
Blue	4.17	0.206	21	4.75	0.174	28		

Table 4.10 : Mean work outputs per unit of metabolic LW for each team in each period with statistical analyses of period differences, together with mean total team work outputs for each period.

Power output

The mean power output /$LW^{0.75}$ of the white team was significantly higher in period 2 once the team had been treated than in period 1 when one of the two animals was infected (Table 4.11). Mean power outputs /$LW^{0.75}$ of the green and striped teams were also significantly higher in the second period than the first, despite the animals being infected during period 2. Those of the other three teams did not differ significantly between periods.

The mean power output /$LW^{0.75}$ of the three teams in group 1 when treated was significantly larger than that of the group 2 teams when they were uninfected, by ANOVA ($F = 13.69$, $P = 0.02$, $df = 1,4$), however the mean power outputs /$LW^{0.75}$ of the two groups did not differ significantly ($F = 0.02$, $P = 0.90$, $df = 1,4$) when both groups were infected. Excluding the two group 1 teams in which only one buffalo became infected, there was no significant difference in the mean power output /$LW^{0.75}$ of the yellow team when infected compared to those of the infected group 2 teams ($F = 0.01$, $P = 0.93$, $df = 1,2$), nor was there a significant difference between the mean power output /$LW^{0.75}$ of yellow when treated and the group 2 teams when uninfected ($F = 5.64$, $P = 0.14$, $df = 1,2$). Finally, within periods 1 and 2 the mean power output /$LW^{0.75}$ of the yellow team and those of the group 2 teams did not differ significantly (period 1, $F = 2.43$, $P = 0.26$; period 2, $F = 0.33$, $P = 0.62$; $df = 1,2$).

Mean total power outputs for each team in each period are given in Table 4.11 to enable comparisons to be made with other studies.

Team	Power output /LW$^{0.75}$ (W/kg$^{0.75}$) Period 1			Period 2			T-tests	
	mean	s.e.	n	mean	s.e.	n	T	P_{period}
Group 1	**Infected**			**Treated**				
White	4.2	0.18	21	4.7	0.15	28	2.04	0.05 *
Red	5.2	0.23	20	4.9	0.15	27	0.81	0.42 ns
Yellow	4.7	0.21	20	4.9	0.16	27	0.99	0.33 ns
Group 2	**Uninfected**			**Infected**				
Green	4.5	0.19	21	5.1	0.16	28	2.29	0.03 *
Stripes	4.2	0.18	21	4.7	0.16	28	2.33	0.02 *
Blue	3.9	0.17	21	4.4	0.16	28	1.95	0.06 ns
Total power output per team (W)								
Group 1	**Infected**			**Treated**				
White	513	21.9	21	618	18.6	28		
Red	643	28.7	20	642	19.1	27		
Yellow	613	26.9	20	689	21.0	27		
Group 2	**Uninfected**			**Infected**				
Green	496	20.9	21	611	18.0	28		
Stripes	513	21.5	21	625	20.1	28		
Blue	553	24.0	21	642	22.0	28		

Table 4.11 : Mean power outputs per unit of metabolic LW for each team in each period with statistical analyses of period differences, together with mean total team power outputs for each period.

4.3.5 Time worked, distance travelled and speed

The time worked and the distance travelled each day by all six teams increased over the first week in both periods and thereafter remained constant with minor day to day fluctuations, although the distance walked did decline towards the end of period 2. The speeds of the teams declined dramatically from day 78 of period 2 until the end of the trial, falling by an average of 41% between day 79 and 88, as illustrated in Figure 4.10 and Figure 4.11. The low working speed of the yellow team on the last working day of period 1 was caused by buffalo 400 going lame. On that day the team had to be withdrawn from work on welfare grounds after walking only 606 m. There was no obvious reason for the slow speed of the blue team on the same day.

There were no significant period differences in the speed of working of any of the six teams (Table 4.12). The green and the stripes teams did however walk significantly shorter distances each day in period 2, when they were infected than they had previously done in period 1 (Table 4.13). The white and yellow teams in group 1 also walked significantly shorter distances in the second work period compared to the first despite being treated with trypanocide before period 2. The time worked by each team each day did not differ significantly between periods with the exception of the time worked by the yellow team which was significantly shorter in period 2 than in period 1 (Table 4.14).

There was a significant negative correlation between the time the green team started work in the morning and the subsequent time worked on that particular day, by least squares linear regression ($R = 0.286$, $P = 0.04$, $n = 51$). The earlier the team started work the longer it was able to continue. None of the other teams exhibited this correlation, nor were there any significant correlations between the LEV parasitaemia of either animal in a team and the time worked by the team. However the working speed of stripes was negatively correlated with the LEV parasitaemia of

Figure 4.10: Daily working speed of each team in group 1 over the experiment

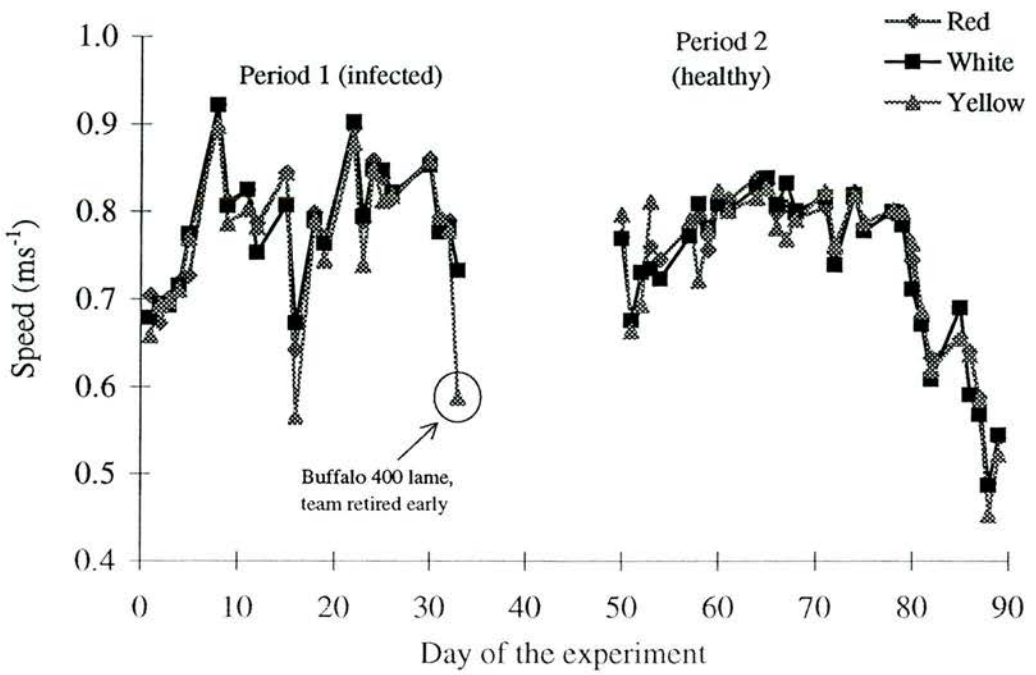
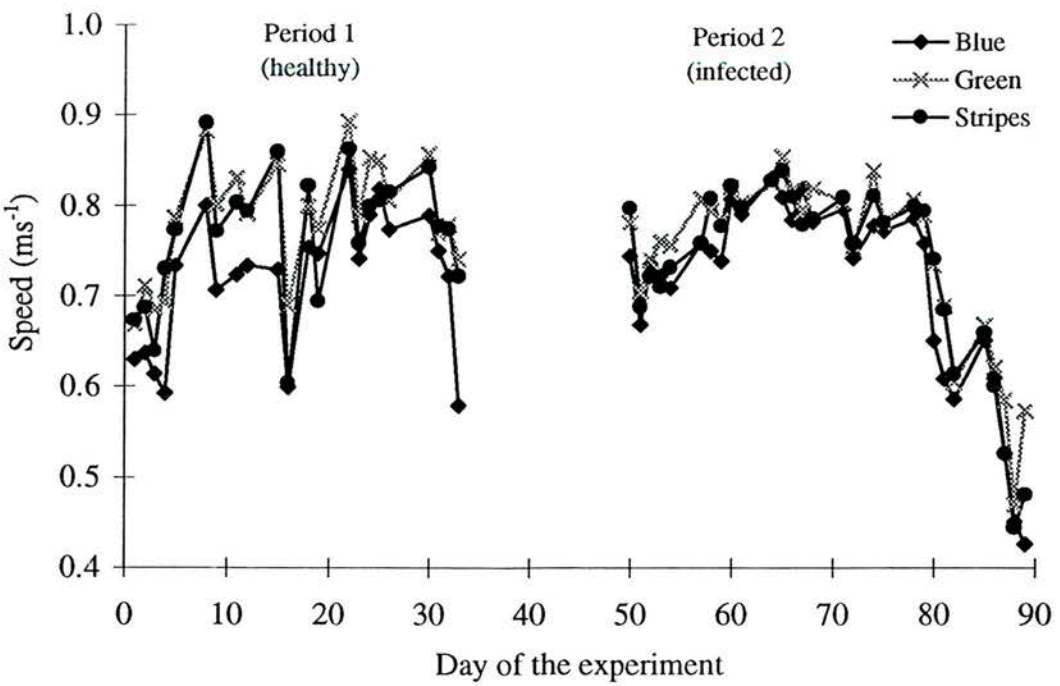


Figure 4.11: Daily working speed of each team in group 2 over the experiment.



Team working speed ($m\ s^{-1}$)								
Team	**Period 1**			**Period 2**			**MW tests**	
	median	**s.i.r.**	**n**	**median**	**s.i.r.**	**n**	**W**	**P_{period}**
Group 1	**Infected**			**Treated**				
White	0.79	0.054	22	0.77	0.063	29	651.5	0.13 ns
Red	0.79	0.058	21	0.77	0.65	28	619.5	0.06 ns
Yellow	0.79	0.053	21	0.78	0.066	28	607.0	0.25 ns
Group 2	**Uninfected**			**Infected**				
Green	0.79	0.055	22	0.78	0.058	29	649.0	0.15 ns
Stripes	0.78	0.050	22	0.78	0.060	29	618.5	0.38 ns
Blue	0.73	0.069	22	0.74	0.065	29	552.0	0.71 ns

Table 4.12 : Median daily working speed for each team in each period with Mann-Whitney tests of period differences.

Daily distance walked by each team (m)								
Team	**Period 1**			**Period 2**			**T-tests**	
	mean	**s.e.**	**n**	**mean**	**s.e.**	**n**	**T**	**P_{period}**
Group 1	**Infected**			**Treated**				
White	6402	219.4	22	5783	187.8	29	2.15	0.04 *
Red	5941	251.5	21	5617	178.4	28	1.08	0.28 ns
Yellow	5840	264.0	21	4874	270.0	28	1.68	0.02 *
Group 2	**Uninfected**			**Infected**				
Green	6270	222.2	22	5585	183.6	29	2.39	0.02 *
Stripes	5899	229.8	22	5255	212.6	29	2.04	0.05 *
Blue	5494	229.7	22	5304	197.8	29	0.63	0.53 ns

Table 4.13 : Mean daily distance walked by each team in each period with T-tests of period differences.

Daily time worked by each team (hh:mm:ss)									
Team	**Period 1**			**Period 2**			**T-tests**		
	mean	**s.e.**	**n**	**mean**	**s.e.**	**n**	**T**	**P_{period}**	
Group 1	**Infected**			**Treated**					
White	2:15:23	2:41	22	2:11:26	3:22	29	0.87	0.39	ns
Red	2:04:45	3:32	21	2:07:31	3:28	28	0.55	0.59	ns
Yellow	2:04:34	3:40	21	1:49:34	4:56	28	2.30	0.03	*
Group 2	**Uninfected**			**Infected**					
Green	2:12:22	3:00	22	2:05:46	3:36	29	1.35	0.18	ns
Stripes	2:07:18	3:05	22	2:00:07	3:39	29	1.44	0.16	ns
Blue	2:06:38	3:19	22	2:04:33	2:51	29	0.48	0.63	ns

Table 4.14 : Mean daily time worked by each team in each period with T-tests of period differences.

buffalo 403 (R = 0.55, F = 11.54, P = 0.002, n = 28) but not with the parasitaemia of 411, the other animal in the team (R = 0.11, F = 0.32, P = 0.58, df = 28). There were no other parasitaemia - speed correlations.

There were no significant differences in the median speeds of the two groups of animals in either period by Mann-Whitney tests (period 1, W = 13.0, P = 0.35; period 2, W = 10.0, P = 1.00; n = 3). Similarly there were no significant differences in the median speeds of the two groups when infected (W = 15.0, P = 0.07, n = 3), nor when group 1 was treated and group 2 was uninfected (W = 10.0, P = 1.0, n = 3). Excluding the two teams in group 1 in which only one of the two animals became infected did not alter these results.

The three group 1 teams travelled significantly further when infected than those in group 2, by ANOVA (F = 11.4, P = 0.03, df = 1,4). There were no other

significant differences in the mean distances travelled by the two groups, neither within periods (period 1, F = 0.37, P = 0.57, df = 1,2; period 2, F= 0.02, P = 0.89, df = 1,4) nor when group 1 had been treated and group 2 was uninfected (F = 1.67, P = 0.27, df = 1,4). There were no significant differences between the groups when the group 1 data was restricted to the results from the yellow team in which both of the buffalo were infected.

The mean time worked by the yellow team in period 2 when the animals had been treated was significantly shorter than the mean time worked by the uninfected group 2 teams in period 1, by ANOVA (F = 1.07, P = 0.03, df = 1,2). There were no other significant differences between the mean times worked by the yellow team and the group 2 teams, nor were there any significant differences between the group 1 and group 2 means when the data for the white and red teams was included in the group 1 dataset.

Team weekly mean times worked and distances travelled are given in appendices 15 and 16 respectively, median speeds in appendix 17.

4.3.6 Estimated energy expenditures for work

The estimated mean net energy expenditures of the buffalo for work (NE_{work}) per unit of $LW^{0.75}$ ranged from 114 to 141 $kJ/kg^{0.75}/d$ or 30 to 37% of the animals' energy requirements for maintenance. There were no significant differences in NE_{work} between periods for any of buffalo, by T-tests (T ranged from 0.15 to 0.99, $P > 0.05$ for all 12 animals). Period means for each animal together with individual T-test results are given in appendix 18.

4.3.7 Buffalo body temperatures

Group 1

Infection had no effect on resting body temperatures as measured at 06.30 h each morning. There were no significant period differences in the 06.30 h body temperatures of the four buffalo that developed patent parasitaemias following infection, by Mann-Whitney tests (P ranged from 0.15 to 0.78). Period medians for each animal with results of the Mann-Whitney tests are given in appendix 19. Infection also had no effect on the number of days when the animals' resting body temperatures exceeded the upper 95% CL of their pre-experiment mean resting temperatures (Table 4.15). There were no significant correlations between 06.30 h body temperature and LEV parasitaemia, by least squares linear regression, R ranged from 0.04 to 0.25, P from 0.16 to 0.84, for the four animals (n = 32).

The two buffalo that did not become infected, 405 and 410, had significantly higher 06.30 h body temperatures in period 2 after they had been treated, than in period 1 ($W = 848.5$, $P < 0.001$ and $W = 993.5$, $P = 0.045$ respectively, $n_1 = 32$ and $n_2 = 40$).

The resting body temperatures of all the animals fluctuated considerably from day to day, irrespective of infection status. In period 2 when the animals had been treated the maximum variation seen was ±0.8°C, in period 1 amongst the infected animals but excluding no. 409, it was ±1.1°C. The resting body temperature of buffalo 409 rose sharply once it contracted anaplasmosis, reaching a peak of 40.4°C on day 30, a rise of 2.7°C on its pre-experiment mean temperature.

The animals' body temperatures rose markedly during the first work session each day, levelling off and in some instances declining slightly during the 20 minutes rest half-way through the working day, before increasing again during the second work session. There were no significant differences in the rates of temperature

Buffalo	Pre-expt. 06.30 h body temperature		Period 1 (infected) 06.30 h body temperature		Period 2 (treated) 06.30 h body temperature		Days examined in each period
	Mean temp. (°C) (n = 18)	Upper 95% CL (°C)	Days above upper 95% CL of pre-expt. mean	% of days examined	Days above upper 95% CL of pre-expt. mean	% of days examined	
402	37.7	37.85	9	38%	16	67%	24
405	37.6	37.86	7	22%	20	63%	32
409	37.7	37.80	22	69%	24	75%	32
410	37.7	37.87	19	59%	23	72%	32
400	37.7	37.91	13	41%	14	44%	32
404	37.7	37.91	22	69%	20	63%	32
Mean ± s.e.+				54.3 ± 8.54		62.3 ± 6.57	
Paired T-test, percentage of days above upper 95% CL in periods 1 (infected) & 2 (treated), T = 1.07, P_{period} = 0.36 ns, n = 4+							

+ Excluding results for buffalo 405 and 410, which failed to develop patent parasitaemias following infection.

Table 4.15 : 06.30 h body temperature parameters for each buffalo in group 1.

change (°C /minute) of five of the six buffalo infected or treated, over any of these three time intervals by Mann-Whitney test. The exception was animal 404 whose temperature rose more rapidly during the first work session when infected than when treated ($W = 603$, $P = 0.02$, $n_1 = 20$, $n_2 = 28$). Median rates of temperature change for each animal over each work session in periods 1 and 2 are given in appendix 20 together with results of the Mann-Whitney tests.

The temperatures of buffalo 400 and 404 taken immediately after work (PW) were significantly higher infected than treated, by Mann-Whitney test.($W = 693$, $P < 0.001$ and $W = 714$, $P < 0.001$, respectively, $n_1 = 21$, $n_2 = 28$) The PW temperatures of the other four animals were not significantly different. The median PW temperature of each animal in each period is given in appendix 22 with results of the Mann Whitney tests of period differences.

Group 2

In this group of animals as in group 1, the resting body temperatures of individuals fluctuated from day to day even when the animals were not infected. There was however a significant rise in the 06.30 h body temperatures of four out of the six buffalo in the infected period compared to the previous period when they were uninfected, by Mann-Whitney tests (W ranged from 779 to 904, $P < 0.01$ for all four animals, $n_1 = 32$ and $n_2 = 40$). There were no significant changes in the resting body temperatures of buffalo 401 and 407 with infection. Median resting temperatures for each animal in each period are given in appendix 19 with results of statistical analyses of period differences. Considering the group as a whole, infection significantly increased the number of days on which the animals' temperatures at 06.30 h exceeded the 95% CL of their pre-experiment mean temperatures (Table 4.16).

Buffalo	Pre-expt. 06.30 h body temperature		Period 1 (uninfected) 06.30 h body temperature			Period 2 (infected) 06.30 h body temperature		
	Mean temp. (°C) (n = 18)	Upper 95% CL (°C)	Days above upper 95% CL of pre-expt. mean	Days examined	% of days examined	Days above upper 95% CL of pre-expt. mean	Days examined	% of days examined
406	37.9	38.13	6	32	19%	21	40	53%
408	37.8	37.98	17	32	53%	34	40	85%
403	37.6	37.84	12	32	38%	24	40	66%
411	37.7	37.92	20	32	63%	34	40	88%
401	37.8	37.92	17	32	53%	19	40	66%
407	37.7	37.85	16	32	50%	25	40	72%
Mean ± s.e.					46.0 ± 6.31			71.7 ± 5.35
Paired T-test, percentage of days above upper 95% CL in periods 1(not infected) & 2 (infected), T = 6.76, P = 0.001***, n = 6								

Table 4.16 : 06.30 h body temperature parameters for each buffalo in group 2.

Once infected the 06.30 h temperatures of the animals rose and fell in concert with their LEV parasitaemias as illustrated in Figure 4.12. There was a strong positive linear correlation between the LEV parasitaemias of four animals, 403, 411 401 and 407 and their 06.30 h body temperatures, tested by ANOVA, R ranged from 0.36 to 0.47, $P < 0.01$, for all four animals ($n = 56$).

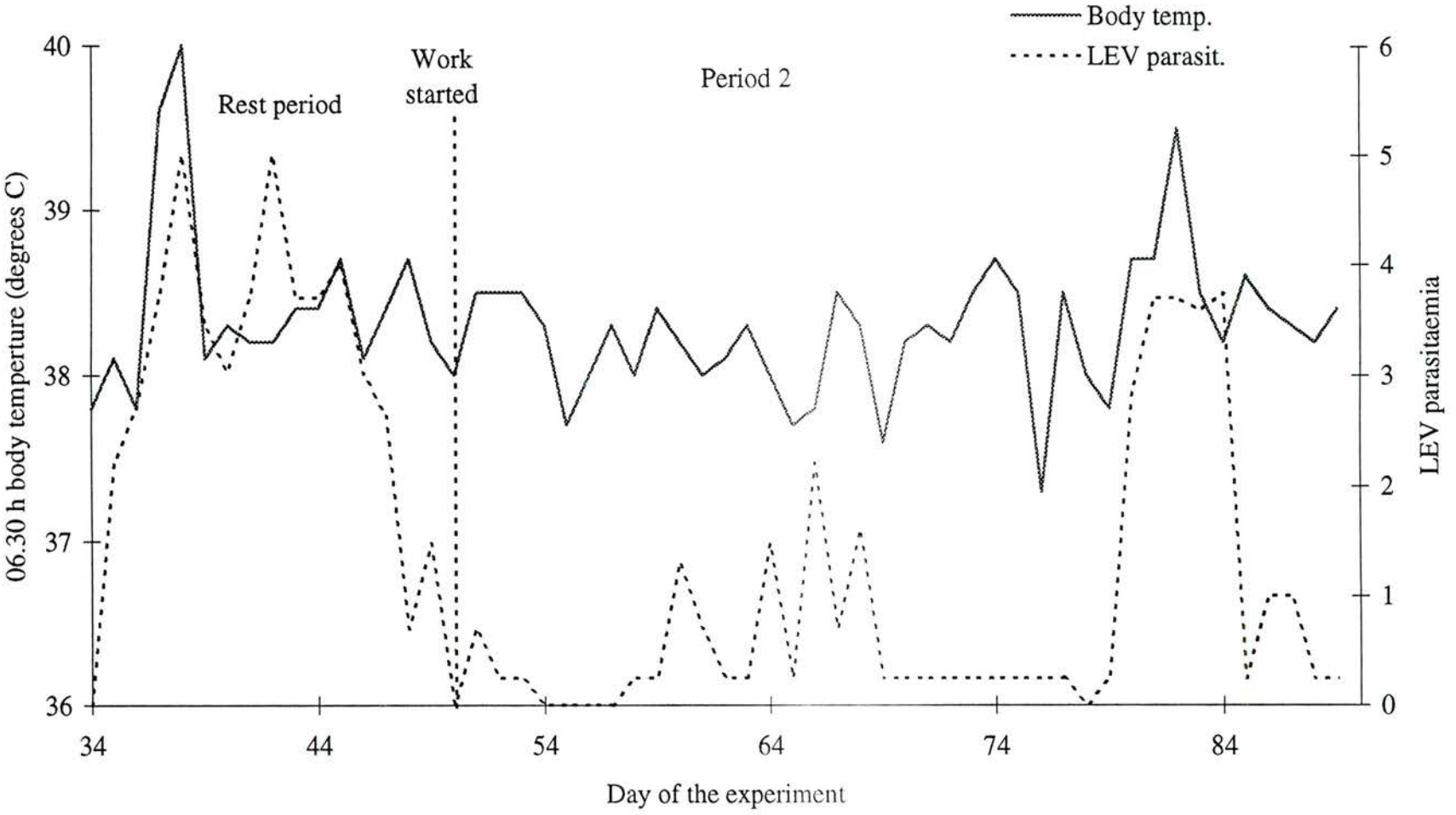
Similar rises in body temperature with work were seen as in group 1, although the rates of temperature change of individual buffalo during each of the three sessions of the working day did not alter significantly once the animals were infected, by Mann-Whitney test (W ranged from 438 to 713, P from 0.052 to 0.89), with the exception of buffalo 411. The temperature of animal 411 rose more rapidly during the first work session and declined more rapidly during rest when not infected ($W = 713$, $P = 0.008$, $n = 29$ and $W = 360$ $P = 0.006$, $n = 28$ respectively). Median rates of temperature change for each buffalo over each work session in each period are given in appendix 21 together with results of the statistical analyses of period differences.

The post-work temperatures of three buffalo, 406, 411 and 401 were all significantly higher when they were not infected compared to when they were infected, by Mann-Whitney tests (P ranged from 0.03 to 0.004, W from 614 to 684, $n = 29$). Period medians for each animal with results of the Mann-Whitney tests are given in appendix 22.

Comparison of group 1 and group 2 temperature parameters

There were no significant differences in the resting body temperatures of the two groups in either period, by Mann-Whitney excluding the data from the two uninfected animals (period 1, $W = 25.5$, $P = 0.50$; period 2, $W = 20.0$, $P = 0.75$; $n_{1,2} = 4, 6$). Similarly there were no significant group differences when the animals were infected ($W = 16.5$, $P = 0.29$, $n_{1,2} = 4, 6$) nor when group 1 was treated and group 2

Figure 4.12: 06.30 h body temperature & LEV parasitaemia of buffalo 411 (stripes) whilst infected.



uninfected (W = 28.0, P = 0.24, $n_{1,2}$ = 4, 6). Excluding the data for buffalo 409 had no effect on these results.

There were no significant group differences in the number of days when animals' resting temperatures exceeded the upper 95% CL of their pre-experiment means, by T-tests, neither within periods (period 1, T = 0.82, P = 0.44; period 2, T = 1.14, P = 0.29; $n_{1,2}$ = 4, 6) nor when infected (T = 1.83, P = 0.10, $n_{1,2}$ = 4, 6) nor when treated / uninfected (T = 1.72, P = 0.12, $n_{1,2}$ = 4, 6). As in the previous analyses, data from the two uninfected animals in group 1 were excluded from the analyses. Removing the data for animal 409 did not alter the significance of any of these results.

4.3.8 Climatic variation

There were no marked changes in climate during the study. Black bulb temperature recorded at the trackside during work did not differ significantly between the two periods, nor did relative humidity at 08:00 h or 09:00 h (Table 4.17) however relative humidity at 10:00 h was higher in period 1 than in period 2. Black bulb temperature rose during the working day, relative humidity tended to fall, although on several days it also increased. Work had to be abandoned once during each period because of rain, on several other days work started late after rain. Because of a fault with the anaemometer no wind speeds were recorded after day 31. Up to that point the mean wind speed recorded on working days was 0.3 ± 0.02 ms^{-1} (n = 70), with a maximum gust of 2 ms^{-1}.

The maximum temperature within the animal house each day was significantly higher in period 2 than period 1 and the diurnal variation was larger, although the minimum temperatures did not differ significantly (Table 4.18).

Time	Period 1			Period 2			T-test
	mean	s.e.	n	mean	s.e.	n	P_{period}
Black bulb temperature (°C)							
08:00 h	26.5	0.26	22	26.5	0.22	28	0.90 ns
09:00 h	29.4	0.28	22	29.4	0.27	28	0.95 ns
10:00 h	30.0	0.29	20	30.3	0.27	28	0.41 ns
Temp rise between 8:00 h & 10:00 h	3.3	0.25	19	3.9	0.30	27	0.21 ns
Relative humidity (%)							
08:00 h	86	0.7	22	85	0.9	27	0.21 ns
09:00 h	84	1.0	22	82	1.1	28	0.27 ns
10:00 h	83	0.9	21	79	1.1	28	0.02 *
RH change between 8:00 h & 10:00 h	− 2.9	0.8	20	− 5.7	0.9	26	0.03 *

Table 4.17 : Mean black bulb temperature and relative humidity at the trackside at hourly intervals during work in periods 1 and 2, with statistical analyses of period differences.

House	Period 1			Period 2			MW test
temperature (°C)	median	s.i.r.	n	median	s.i.r.	n	P_{period}
24 h max.	31.5	0.25	24	32.5	0.75	39	0.04 *
24 h min	24.0	0.50	24	24.0	0.25	39	0.50 ns
Diurnal variation	7.6	0.56	24	8.5	0.75	39	0.02 *

Table 4.18 : Median maximum and minimum temperatures within the buffalo house during each period with median diurnal variations in each period.

4.3.9 Animal liveweight changes

Group 1

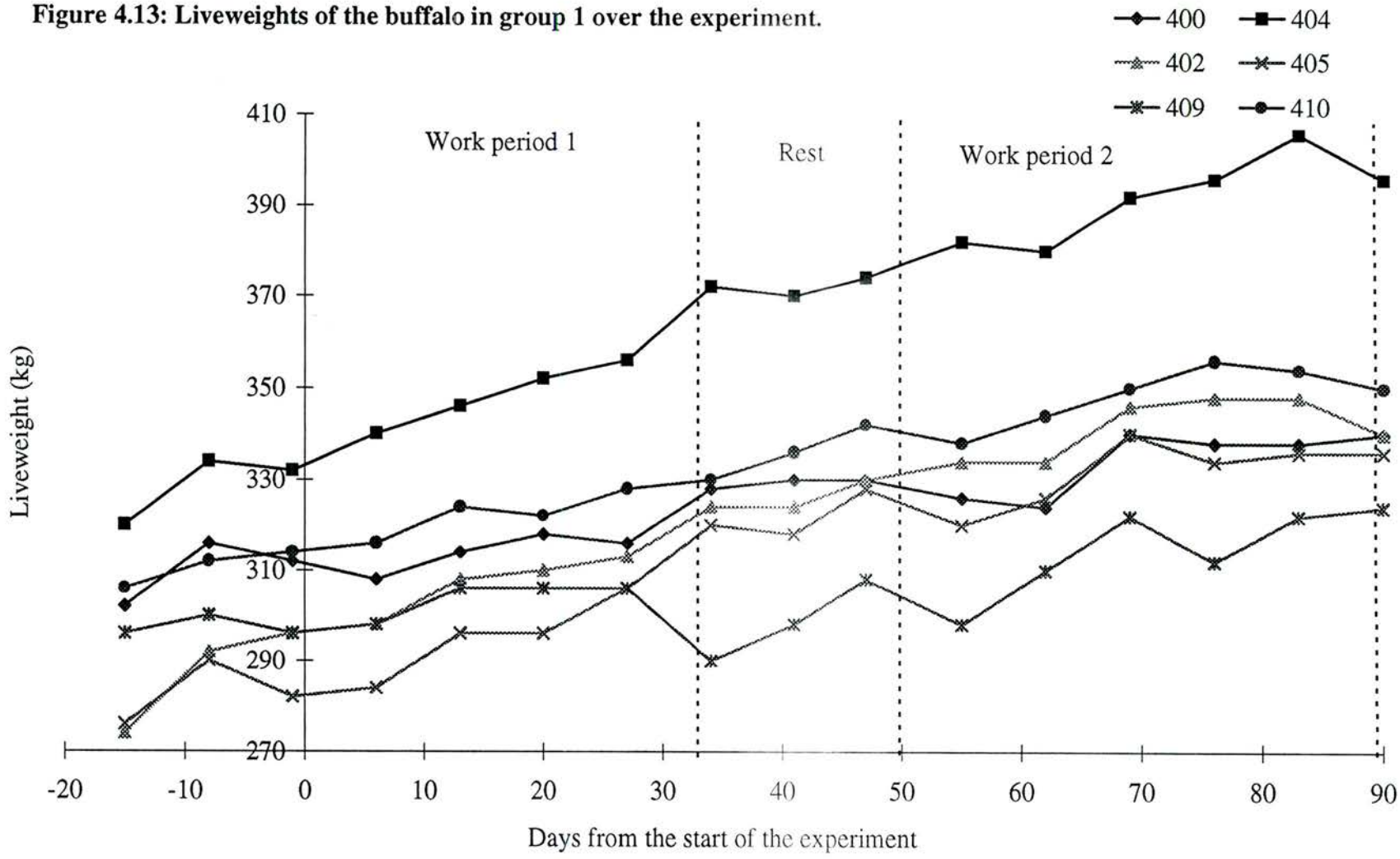
Five of the six animals in group 1 gained weight throughout the experiment, the exception was the buffalo with anaplasmosis, 409 (Red) which lost weight over the first work period but gained weight over the second (Figure 4.13). The rates of LWG of the five animals which gained weight consistently, were significantly higher in the work period when they were infected than in the period after treatment (Table 4.19). The mean LW of all six buffalo at the start of the experiment (day −1) was 305 ± 7.2 kg with the animals gaining an average of 43 kg over the experiment. Liveweights for each animal over the experiment are given in appendix 23.

Buffalo no.	Period 1 (infected) LWG (kg/day)	Period 2 (treated) LWG (kg/day)
402	0.76	0.36
405	1.04	0.32
409	−0.02	0.49
410	0.47	0.34
400	0.44	0.34
404	1.04	0.65
Mean ± s.e. (excluding 409)+	0.75 ± 0.131 (n = 5)	0.40 ± 0.062 (n = 5)
Paired T test comparing LWG in periods 1 & 2, T = 3.10, P = 0.036 *, n = 5		

\+ Buffalo 409 excluded because of concurrent anaplasma infection.

Table 4.19 : The rate of liveweight gain (LWG) of each buffalo in group 1 over each period calculated by regression analysis (n = 6) with results of a paired T - test comparing LWG in each period.

Figure 4.13: Liveweights of the buffalo in group 1 over the experiment.



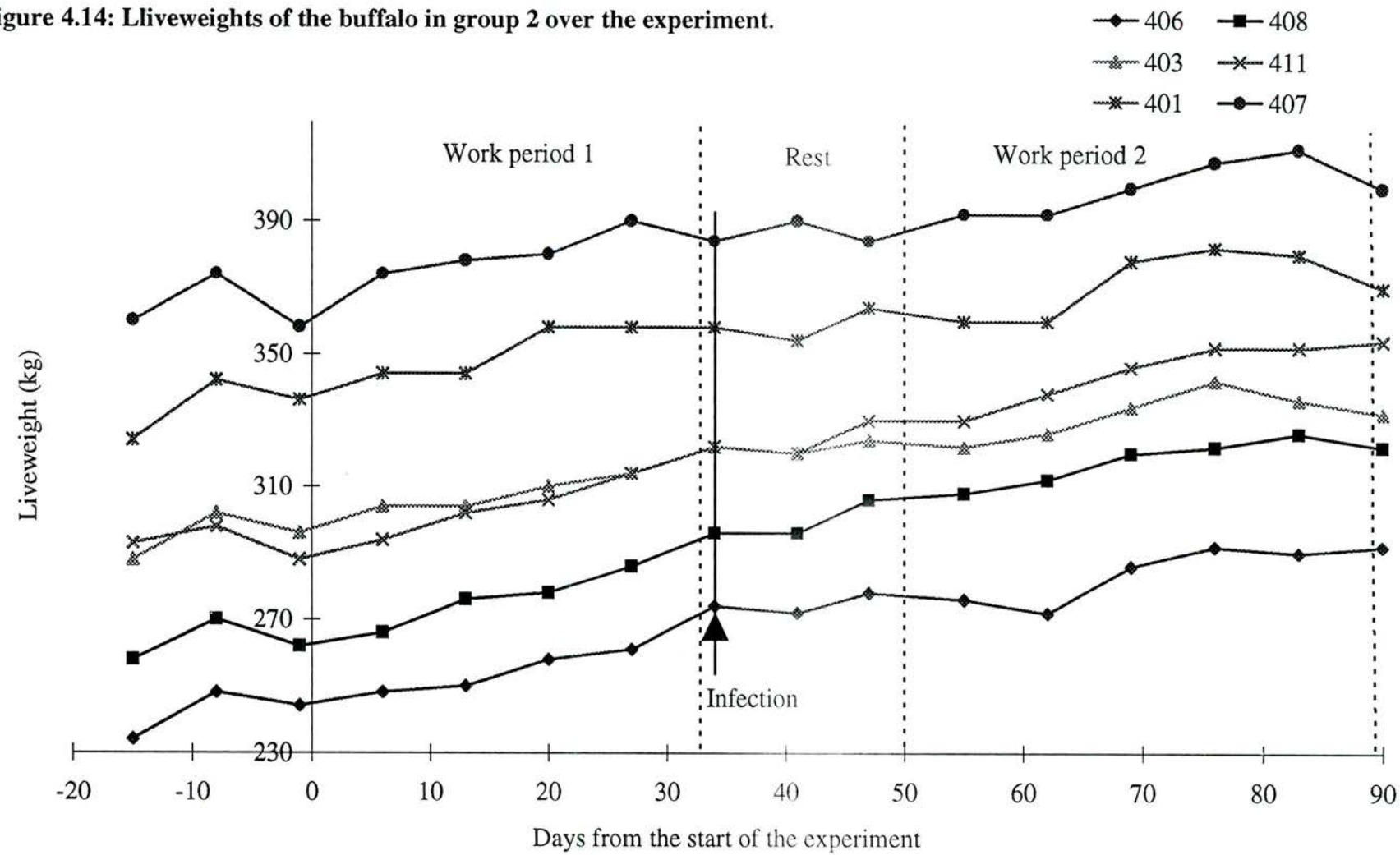
Group 2

All the buffalo in this group gained weight consistently throughout the experiment (Figure 4.14). The rates of LWG of the animals were significantly higher in period 1 when they were not infected compared to period 2 when they were infected (Table 4.20). The mean LW of the six animals before the study (day −1) was 297 ± 17.7 kg with an average weight increase of 48 kg over the experiment. Individual weights are given in appendix 23.

Buffalo no.	**Period 1 (not infected) LWG (kg/day)**	**Period 2 (infected) LWG (kg/day)**
406	0.80	0.45
408	0.95	0.47
403	0.68	0.34
411	0.96	0.65
401	0.68	0.40
407	0.73	0.52
Mean ± s.e.	0.80 ± 0.052 (n = 6)	0.47 ± 0.044 (n = 6)
Paired T test comparing LWG in periods 1 & 2, T = 8.96, P < 0.001 ***, n = 6		

Table 4.20 : The rate of liveweight change (LWG) of each buffalo in group 2 over each period calculated by regression analysis (n = 7) with results of a paired T -test comparing LWG in each period.

Figure 4.14: Lliveweights of the buffalo in group 2 over the experiment.



A comparison of group liveweight changes.

The mean rates of LWG of the buffalo in groups 1 and 2 excluding animal 409, did not differ significantly in period 1, by T-test ($T = 0.38$, $P = 0.71$, $n_{1,2} = 5, 6$). Similarly there was no significant difference in the rates of LWG of the two groups in the second period ($T = 0.94$, $P = 0.37$, $n = 6$). Excluding the two animals which did not become infected from the group 1 dataset, did not alter the significance of either of the tests. The group mean liveweights of the buffalo were not significantly different at the start of the experiment, nor at the end, by T-tests ($T = 0.42$, $P = 0.68$ and $T = 0.14$, $P = 0.89$ respectively, $n = 6$).

4.3.10 Nutrition

Feedstuffs offered

The chemical composition of the concentrate fed to the animals did not vary significantly between periods, however the DM, GE, CP and fibre content of the elephant grass were all significantly higher in period 2 compared to period 1 (Table 4.21). Both the concentrate and the grass proved to be of a higher quality than was originally estimated when the rations were formulated.

Feed intakes

Three animals in group 1 and two in group 2 intermittently refused part of their concentrate during the first work period, however there was no pattern to these refusals, with the buffalo leaving a large proportion of their concentrate ration one day and then consuming all of it the next. The refusals of the group 1 animals were not associated with parasitaemic episodes. During period 2 only one animal in group 1 left any concentrate and then only on the first day of work, but not thereafter.

Chemical composition	Concentrate						Elephant grass					
	Period 1		Period 2		T-test		Period 1		Period 2		T-test	
	mean	s.e.	mean	s.e.	T	P_{period}	mean	s.e.	mean	s.e.	T	P_{period}
DM (%)	89.6	0.08	89.6	0.20	0.02	0.99 ns	18.1	0.47	22.3	0.46	6.18	< 0.001 ***
GE (MJ/kg DM)	18.0	0.16	18.4	0.08	2.03	0.07 ns	17.5	0.07	18.2	0.24	2.48	0.035 *
CP (g/kg DM)	16.7	0.65	17.3	0.34	0.75	0.47 ns	7.7	0.64	11.3	0.48	4.55	0.001 ***
NDF (g/kg DM)	32.6	0.70	31.0	0.38	2.09	0.07 ns	70.8	0.44	74.4	0.52	5.14	< 0.001 ***
ADF (g/kg DM)	12.4	0.33	11.9	0.44	0.80	0.45 ns	40.1	0.68	45.4	0.22	7.95	< 0.001 ***
Ash (g/kg DM)	8.3	0.41	7.9	0.15	1.07	0.31 ns	10.4	0.37	11.8	0.07	3.81	0.004 **
n	5		6				5		6			

Table 4.21 : Chemical analyses of the feedstuffs offered in each period with T-test results comparing period means.

Some of the refusals were considered to be due to contamination following spillage rather than loss of appetite.

The mean elephant grass intakes of the animals in group 1 expressed as percentages of grass offered, did not differ significantly between periods, by paired T-test (T = 2.22, P = 0.08, n = 6), the overall mean intake was 93 ± 1.1%. The mean elephant grass intake of the group 2 animals was just significantly higher in period 2 than period 1 (T = 2.58, P = 0.05, n = 6). In period 1 the mean intake of the group 2 animals was 89 ± 2.1% of the grass offered, compared to 92 ± 2.2% in period 2.

The mean gross energy (GE) and crude protein (CP) intakes of both groups of animals per unit of metabolic liveweight ($LW^{0.75}$), were significantly higher in period 2 of the experiment than in period 1 (Table 4.22). Within each period however, there were no significant differences in the mean GE intakes per unit of $LW^{0.75}$ of the two groups of animals, by T-test (period 1, T = 0.06, P = 0.95; and period 2, T = 0.41, P = 0.69; n = 6) nor in the CP intakes per unit of $LW^{0.75}$ (period 1 T = 0.47, P = 0.65; and period 2, T = 0.45, P = 0.66; n = 6). Period mean GE and CP intakes per unit $LW^{0.75}$ for individual buffalo are given in appendix 25.

	Gross energy ($MJ/kg^{0.75}$)					
	Period 1		Period 2		Paired T-test results	
Group	mean	s.e.	mean	s.e.	T	P_{period}
1	1.66	0.027	1.96	0.021	8.30	< 0.001 ***
2	1.66	0.042	1.98	0.037	9.64	< 0.001 ***
	Crude protein($g/kg^{0.75}$)					
1	10.4	0.22	14.4	0.14	12.77	< 0.001 ***
2	10.6	0.18	14.6	0.26	24.01	< 0.001 ***

Table 4.22 : Mean period gross energy and crude protein intakes per unit of animal metabolic liveweight for each group, with paired T-tests comparing period means (n = 6).

4.4 Discussion

T. evansi infection had no significant effect on the work output of either group of buffalo in this study, although the outputs of both groups of animals did fall sharply towards the end of the second work period (between day 79 and 88), commencing 45 days after the group 2 animals were infected. This decline in work perfomance coincided with marked rises in the parasitaemias of three of the group 2 animals, one from each team. These two events, namely the decline in work output and the increase in parasitaemia amongst the infected group 2 buffalo may have been linked, however this cannot be proven, since the output of the treated group 1 animals also declined at this time. Furthermore, there was no decline in work output during period 1 when the group 1 animals were infected. If the decline in work performance observed towards the end of period 2 was related to the *T. evansi* infections in the group 2 buffalo, the absence of a similar decline during period 1 may have been due to the different parasite strains used to infect the two groups of animals. The effects of strain differences in infectivity and pathogenicity have been seen in the field. Wells (1981) reported outbreaks of clinical trypanosomosis when buffalo which were already infected with *T. evansi* were exposed to different parasite strains. Luckins (1983) proposed that differences in the pathogenicity of *T.evansi* isolates might be explained by differences in their virulence. The decline in the work output of the treated group 1 animals observed at the end of period 2 might be attributable to a peer effect, whereby all six teams worked at the speed of the slowest infected team because of the small track used for the study, however this remains conjecture.

In a ten day period from day 79 to 88, the speeds of the three group 2 teams fell by an average of 41%, with work output falling by 40% and power by 30%, over the same time interval. This fall in work output is slightly larger than that reported by Rukmana (1979) who found that buffalo experimentally infected with *T. evansi* were

able to plough only 153 m^2 of wet soil in one hour compared to 216 m^2 ploughed by uninfected animals, a fall in work output of 29%. The reduction reported by Rukmana however appears to be based on only one hour's ploughing before infection and one hour several weeks after infection. Unfortunately the author provided few details of the study and it is not known how many teams of animals were used. If Rukmana's animals had been worked for longer after infection the fall in work output might have been greater.

As previously stated the fall in the work output of the three teams in group 2 coincided with marked rises in the parasitaemias of three animals, one from each team. The parasitaemia of a fourth animal increased some five days later. If the two factors are related, and this remains pure speculation, it is not clear whether the reduction in output was caused by sick animals with high parasitaemias being less able to work, or whether the increases in parasitaemia were a result of work fatigue suppressing buffalo immune responses, with a consequent negative feedback on work output. Payne *et al* (1991a) reported that exercise did not exacerbate the effects of *T. evansi* in experimentally infected buffalo, however Löhr *et al* (1985) and Wells (1981) have documented outbreaks of disease associated with exercise or work in the field. In cattle and horses even very short periods of intense exercise have been shown to depress immune responses and increase susceptibility to disease (Anderson *et al*, 1991; Wong *et al*, 1992). In humans there is evidence that the immunosuppressive effects of intensive exercise may be cumulative (Lewicki *et al*, 1987; Mackinnon *et al*, 1988; Tomasi *et al*, 1982). This led Fitzgerald (1988) to propose that although moderate exercise appears to enhance immune responses in man, intense exercise may actually cause immunosuppression.

The parasitaemic peaks that coincided with the decline in work output of the group 2 teams occurred late in the infections; 45 days after infection, and over 30 days after the initial post-infection parasitaemic peaks. During the intervening 30 days

the parasitaemias of all six animals in the group remained very low with only two developing parasitaemias in excess of 10 trypanosomes /field, each one on a single occasion. This pattern of an initial rise in parasitaemia immediately after infection followed by a long interval with few parasites evident in the blood, then a second rise in parasitaemia some 45 days after infection may be typical of Garut 197 the *T. evansi* isolate used. Alternatively the late rise in trypanosome numbers may have been triggered by cumulative work fatigue depressing host immune responses. In a similar experiment to this one, Payne *et al* (1991a) found that *T. evansi* (Garut 655) which was originally isolated from the same area of Central Java, Indonesia as the strain used here, produced parasitaemic peaks every seven days with a similar pattern in both exercised and rested buffalo. A further experiment is required to compare the typical parasitaemia profiles of Garut 197 in worked and unworked buffalo.

Following their studies and in the light of evidence from other scientists, Payne *et al* (1991a) suggested that the work output of anaemic animals may be restricted by the reduced oxygen carrying capacity of their blood, that of pyretic animals by their inability to tolerate further rises in body temperature, or by the extra energy demands of an increased metabolic rate resulting from the elevated body temperature. Previously Rukmana (1979) had suggested that PCV was significantly correlated to work output in swamp buffalo, postulating that a fall in PCV such as occurs *with T. evansi* infections would lead to a decline in work output through a reduction in the oxygen carrying capacity of the blood. Supporting this, Pearson (1989b) found that the power output of a pair of anaemic but otherwise apparently healthy buffalo in Nepal was 33% less than that of a healthy control pair after only 3 days work, completed over an 18 day period. Furthermore, buffalo are acknowledged to be particularly prone to heat stress when exercised being poor thermoregulators due to their reduced sweating ability compared to cattle (Moran, 1973). Consequently an animal starting work with an elevated body temperature will reach a critical

temperature beyond which it is unwilling to continue working, more rapidly than one starting with a normal body temperature, assuming that the temperatures of both individuals rise at the same rate. Stephen (1986) calculated that for every 1°C rise in an animal's body temperature its metabolic rate rises by approximately 15%, leading Payne *et al* (1991a) to speculate that this might result in a decrease in the energy available for work. Results from this study appear to support the hypothesis that anaemia or pyrexia may reduce work output. The rapid increases in the parasitaemias of four animals observed at day 78 as the work output of the teams declined, were accompanied by sharp falls in the PCV's of three of the four animals. Furthermore the resting body temperatures of three of those animals were positively correlated with their parasitaemias, high parasitaemias coinciding with high body temperatures. Ambient temperature and relative humidity were monitored at the trackside on working days throughout this study to ensure that any changes in buffalo work performance were caused by infection and not by changes in the climate.

On the last work day of this study the speeds and work outputs of the group 2 teams actually recovered slightly. The next logical step would therefore be to continue working the animals for longer to investigate whether the increase in parasitaemias and depression in work outputs observed around day 78 was merely transitory, or part of a longer term pattern.

The significant increases in the power outputs /$LW^{0.75}$ of two of the three group 2 teams recorded in period 2 compared to period 1, were the result of increases in the average draught forces (ADF) required to pull the sledges caused by changes in the track surface. They do not imply that infection increased power output. This was confirmed by the increase in the ADF as a percentage of team LW, required by the blue team pull to their sledge in period 2 compared to period 1, despite the sledge load remaining at 25% of team LW. Furthermore the work outputs /$LW^{0.75}$ and speeds of all three teams did not change significantly between periods. The changes

in the track surface over the course of the trial illustrate the importance of monitoring ADF regularly, preferably daily in a study of this type.

Despite fluctuations, the draught force required by the blue team to pull their sledge remained within similar limits to those recommended by Goe and McDowell (1980) and by Martin and Teleni (1989) as sustainable for buffalo walking at between 0.66 and 1.1 m s^{-1}, namely 10 - 14% of LW with a 7.5% reduction for multiple hitching of animals. The estimates of work done by the buffalo and their consequent net energy expenditures were comparable to values from other studies for cattle and buffalo (Payne *et al*, 1991a; Pearson, 1989a; Pearson *et al*, 1989) although the animals were probably worked for a shorter time each day than most farmers' animals (Bamualim, Basuno, Liem, Hidayat and Peacock, 1995; Teleni, 1993).

The sledges were all loaded to a fixed proportion of team liveweight for the duration of the study to ensure that each team was working at a similar percentage of its maximal power output assuming they all walked at a similar speed; draught power output being positively correlated to animal liveweight (Starkey, 1989). Had mature adults been used for the study instead of younger animals which were still growing sledge load adjustments might have been avoided. Young animals were however used in preference to adults, as they were less likely to have had much work experience which could have influenced the work performance of individuals differentially, and more importantly they were more likely to be immunologically naive to the infecting trypanosomes.

The work performance data obtained from the teams in group 1 when they were infected was difficult to interpret because of the poor infection rate achieved, which resulted in infected and uninfected animals working together on a single yoke. During period 1 the only team in which both animals became infected was the yellow team. This shortage of data for comparison, coupled with the normal variation in work output between teams, might explain why there were no significant group

differences in any of the work output parameters in period 1. Unfortunately working the buffalo separately rather than in pairs was impractical because of the short track length and the cost of doubling the labour force to enable all 12 animals to be worked simultaneously.

There was no obvious rise in the parasitaemias of the group 1 animals towards the end of the infected period (1), nor was there a decline in team working speeds and work outputs, as was observed with the group 2 teams. Pursuing the hypothesis that infection and work output may be interconnected there are a number of possible explanations for the group 1 results. The animals in group 1 were only worked for 33 days after infection, whereas the decline in the work performance of the group 2 animals was not seen until 45 days p.i., 30 days after work had commenced. Consequently it is possible that the animals in group 1 were not infected for long enough for infection to depress work output. It is more likely however that the different parasitaemia and consequent work performance patterns seen in the two groups of animals were due to differences in the pathogenicity of the two strains of *T. evansi* used; Bakit 362 used to infect group 1 certainly appeared less infective than Garut 197 used to infect group 2. If so, it may be important to look at the strain characteristics of a parasite when attempting to assess its effect on the work performance of draught animals.

It was originally envisaged that a single *T. evansi* isolate would be used to infect both groups of buffalo in this study, with the expectation that most if not all of the animals would become parasitaemic as previous experience had suggested (Payne *et al*, 1991a). Because of the small number of buffalo in each group and the fact that they were to be worked in pairs, it was important that a high proportion of them developed patent parasitaemias to quantify any effects of infection on work output. Consequently following the low infection rate achieved in group 1 with only four animals becoming parasitaemic despite two inoculations, a different isolate known

from previous work to be more pathogenic (Payne, 1991), was used to infect group 2. As a further precaution to ensure that all animals were parasitaemic by the time work commenced, the group 2 animals were infected two weeks before the start of period 2, rather than immediately before work as happened with group 1.

The difference in the infectivity of the two trypanosome isolates used was probably caused by inherent strain differences, although it may have been due to poor inoculation technique leading to few viable trypanosomes entering the hosts' blood, or to differences in the susceptibilities of the animals in the two groups. The latter is however unlikely as animals were allocated to the two groups at random and none had antibodies to *T. evansi* by ELISA at the start of the experiment. It is also possible although improbable, that the two buffalo in group 1 which were apparently aparasitaemic throughout period 1 were in fact infected, but that the small numbers of trypanosomes present were simply not detected by MHCT. Using MHCT parasites were detected in the other four animals infected with the same isolate, at least 30% of the time.

Although the trypanocide treatment used to rid the group 1 animals of infection was successful in that no trypanosomes were detected in blood samples from these animals during period 2, the speeds and work outputs /$LW^{0.75}$ of all three teams declined markedly from about day 78 following a similar pattern to that seen in the infected group 2 teams. This decline in work output occurred at a time when the treated group 1 animals might have been expected to be getting fitter following a month's work. Pearson (1989a) recorded improvements in the work output of swamp buffalo over just six days work as animals got fitter, although the buffalo in that study were not recovering from *T. evansi* infections. It is possible that the day 78 decline in work performance seen in the infected animals in this study was not related to increases in parasitaemia, but was simply a period effect on both the treated and the infected groups, caused by changes in nutrition, climate, track condition, driver

motivation or even a peer effect between teams. As the gross energy and crude protein intakes of all twelve buffalo did not differ significantly between periods and similarly there were no significant climatic differences observed between periods with the exception of an increase in relative humidty at 10.00 h in period 2, the first two variables can be discounted as plausible explanations. Changes in track conditions towards the end of period 2 increasing ADF's and making the animals work harder to pull the sledges were also not the cause; the ADF required by the blue team to pull their sledge from day 78 onwards was not as high as had been recorded earlier in period 2. The conclusion is therefore that the general decline in the work performance of all teams observed towards the end of period 2 was probably caused by a peer effect, which might have been driven by a fall in the work performance of one or more of the infected teams in response to rising parasitaemias in one or more animals, however this remains unproven. This may or may not have been compounded by a fall in driver motivation as the end of the experiment approached; the drivers were local farmers hired as casual labour for the duration of the study only.

The working speeds of all six teams each day were broadly similar throughout the experiment, towards the end of period 2 they were almost identical. The short track used for the experiment meant that each team could generally see at least one other and that convoys often formed behind the slowest pair, despite drivers being instructed to overtake when necessary. Furthermore during the study the animals very soon became accustomed to the daily routine, consequently towards the end of each working day as the first teams were retired, those still working became increasingly difficult to control as they saw their contemporaries departing. These peer effects have been observed in other similar studies (Roberts, 1993). Recognising these potential problems, the original experimental design envisaged using a much longer track allowing pairs of animals to be worked in relative isolation. Unfortunately the land identified for the track was ultimately not available. The short track and consequent monotony of the work undoubtedly had a demotivating effect

on the drivers. Pearson (1989b) used a 16.4 km circuit in an experiment which demonstrated reductions in the work output of anaemic buffalo.

In future studies of this type any circuit used must be long enough to ensure that animals or teams being worked simultaneously are kept apart. Animals or teams should also be started at intervals and at random each morning to minimise the possibility of longer term peer effects developing. Because of the very short track no attempt was made to randomise the order in which the teams started work each morning in this study. Ensuring driver motivation during a long draught animal experiment is difficult.

Emaciation and weight loss are common symptoms of chronic trypanosomosis in buffalo (Dieleman, 1986; Löhr *et al*, 1985) which have been reported to occur without loss of appetite (Lingard, 1899) consequently food intakes and animal liveweights were monitored throughout the study. The higher rates of animal liveweight gain observed in period 1 compared to period 2 were probably caused by compensatory growth in period 1. Prior to the experiment many of the buffalo had to be starved for a short time to encourage them to eat their concentrate rations as most of them had not eaten such food before. This caused small transient weight losses. Unfortunately the acclimatisation period before the experiment was probably too short to allow rates of liveweight gain to stabilise again following starvation, hence the slightly higher rates of liveweight gain in period 1 compared to period 2.

The work performance of draught animals is known to vary between teams worked under similar conditions (Pearson *et al*, 1989) affected by a plethora of different factors. To accurately quantify any changes in work performance due to *T. evansi* infection, this study was designed to allow each animal or team to act as its own control, with only limited comparisons being made between teams. Two groups of animals were used in a crossover design, simply to monitor any period differences in work performance which might have occurred due to changes in factors such as

climate, animal nutrition, driver motivation and animal fitness or fatigue. The alternative to this design would have been to work two much larger groups of animals simultaneously for a single period, one group infected the other not. This was not a feasible option, neither logistically nor financially.

In conclusion, this study has suggested that there may be a link between trypanosomosis and work output in draught buffalo, although this remains unproven. It has however illustrated some of the difficulties associated with demonstrating and quantifying any such interaction. It has highlighted the large number of variables that can affect the work output of draught animals, as well as illustrating the problems involved in attempting to work a large number of animals simultaneously, but independently, under exactly the same conditions. In future studies it may be easier to give all animals a fixed amount of work to do and then monitor how that work is performed by different animals on different experimental regimes. The difficulty arises in fixing the amount of work to provide a similar stressor to that experienced by farmers' animals working in the field. Further studies are required to prove a link between disease and work.

5. THE EFFECTS OF EXERCISE AND PLANE OF NUTRITION ON SHEEP IMMUNE RESPONSES

5.1 Introduction

In many tropical farming systems cultivation operations must be carried out promptly at the start of the rainy season to allow the growing crops to make maximum use of soil moisture. In such situations, the demand for draught animal power is sporadic over the year and is often greatest at the time when feed resources are scarce and consequently animals are in poor condition. The combined stressors of work and under nutrition conspire to make draught animals very vulnerable to disease precisely when they are needed most. The effects of stress on immune function have been extensively studied in a wide variety of animal species however the results are equivocal with some experiments suggesting stress depresses immune responses, others that it actually enhances them (Griffin, 1989; Kelley, 1980). Neurophysiological responses to stress vary quantitatively and qualitatively depending not only upon the nature of the stressor, but also on the genetic background, age, experience and management of the animal being stressed (Griffin, 1989). Consequently the same stressor may have different immunological consequences for different animals.

Stressful exercise on a treadmill has been demonstrated to make cattle more susceptible to experimental pneumonic pasteurellosis (Anderson *et al*, 1991). Levinson, Milzer and Lewin (1945) found that monkeys exercised whilst incubating poliomyelitis had a higher incidence of more severe paralysis than rested controls. Similarly mice forced to swim whilst infected with Coxsackie B virus had a mortality

rate of 50% compared to only 5% in the control group (Gatmaitan *et al*, 1970). In man there is increasing evidence that acute or severe exercise such as that undertaken by serious athletes can lead to post-exercise immunodepression, with overtraining increasing susceptibility to infection (Fitzgerald, 1991). Tomasi *et al* (1982) found that at the end of the ski season members of the US National Ski team had significantly lower resting salivary IgA levels than control subjects and that these levels dropped still further after 2 - 3 hours exhaustive exercise. Conversely in laboratory mice, exercise based on running has been shown to increase antibody production (Liu and Wang, 1987) and inhibit tumor growth (Good and Fernandes, 1981). Pedersen (1991) reviewing his own work and that of a number of other scientists concluded that human immune responses are enhanced during both moderate and severe exercise and that only severe exercise leads to post-exercise immunodepression.

The link between exercise and immunity in draught animals is not clearly understood. Munzinger (1982) and Starkey (1981) have speculated that increased secretion of corticosteroids and catecholamines induced by stressors such as work or exercise (Stephens, 1980; Tizard, 1992), may make draught animals more susceptible to disease. Plasma cortisol levels in cattle have been shown to rise in response to forced exercise (Arave, Walters and Lamb, 1978).

Corticosteroids and catecholamines are known to have immunomodulatory effects (Griffin, 1989) altering the distribution of blood mononuclear cells which in turn causes changes in the composition of cell subsets and in immune responses (Pedersen, 1991). The administration of glucocorticoids has been shown to increase susceptibility to disease as well as activating latent infections in a number of animal species (Griffin, 1989). Cattle injected with glucocorticoids become less resistant to a variety of diseases including coccidiosis (Niilo, 1970; Stockdale and Niilo, 1976), bovine viral diarrhoea (Shope *et al*, 1976) and babesiosis (Callow and Parker, 1969).

The timing of any increase in plasma glucocorticoids relative to an immunological challenge, can however have a profound effect on the subsequent immune response. Roth and Kaeberle (1983) found that administering glucocorticoids to cattle simultaneously with bovine viral diarrhoea vaccine actually enhanced resistance to a subsequent disease challenge. In tumor rejection studies in rats Riley (1981) demonstrated that administering glucocorticoids 3 - 7 days before challenge increased the rate of rejection whereas administration 4 - 6 days after tumor implantation decreased rejection rate. Croiset, Veldhuis, Ballieux, De Wied and Heijnen, (1987) suggested that the magnitude of any increases in plasma glucocorticoids may also have a profound effect on subsequent immune responses, with moderate increases enhancing responses rather than suppressing them.

Outbreaks of disease often occur in times of famine, however it would be wrong to assume that malnourished animals are necessarily more susceptible to infection than well fed ones. An increase in the incidence of a disease during a period of famine may be attributable to favourable environmental conditions for the multiplication and or transmission of the pathogen rather than to reduced host resistance to infection. The effects of malnutrition on immune function are complex and although bacterial infections can thrive in malnourished host tissue, viruses usually require healthy cells in which to replicate (Tizard, 1992). The type of malnutrition is important in determining the likely effects on the immune system. Protein deficiencies can directly impair immunoglobulin production (Cooper, Good and Mariani, 1974) while mineral and vitamin deficiencies and imbalances have specific effects on different components of the immune system. Deficiencies of vitamin A, B_{12} and folic acid for example can all depress cell-mediated responses, vitamin E deficiency in dogs and lambs reduces lymphocyte blastogenesis, vitamin D is vital for proper macrophage function (Tizard, 1992). Severe starvation *per se* causes rapid thymic atrophy leading to a reduction in circulating T-cell numbers and

function, which in turn depresses cell-mediated immunity, however it has little effect on B-cell function which controls humoral immunity and in particular immunoglobulin production (Tizard 1992). In starving animals serum immunoglobulin levels of all isotypes together with secretory IgE levels tend to remain normal or rise slightly, whereas secretory IgA generally falls.

Both cell-mediated and humoral immune responses are important in parasitic infections (Cox and Liew, 1992) although their significance varies with the infecting organism. Immune responses to most parasites involve considerable overlap between B cell and T cell mediated pathways. Cell-mediated responses are of primary importance in controlling viral diseases (Mims, 1985), whereas bacterial infections elicit antibody responses, although phagocytosis and intracellular destruction by macrophages are also important in their control (Winter, 1979). Antibodies are of primary importance against extracellular parasites living in the bloodstream such as trypanosomes and extra-cellular stages of protozoal infections like leishmania and toxoplasmosis caused by *T. gondii*, while cell-mediated responses are predominant in the control of intracellular protozoal stages (Reiner and Locksley, 1993; Tizard, 1992). Helminth and arthropod ectoparasites and their vectors can induce cell-mediated immune responses as well as stimulating the production of IgG and IgE antibodies.

The purpose of the study described below was to examine the effects of prolonged moderate exercise and under-nutrition such as might be experienced by draught animals in the field, on both cellular and humoral immune responses of sheep under controlled conditions. The sensitisation techniques and the assays used to monitor cellular and humoral responses were adapted from techniques used in other experiments to demonstrate immunosuppression in sheep infested with *Amblyomma variegatum* ticks (Lloyd and Walker, 1993) reported in detail by Lloyd (1993) and in sheep infected with chronic dermatophilosis (Ellis, Sutherland and Gregory, 1989).

The antigens chosen to stimulate immune responses were two of those used by Lloyd and Walker (1993).

The aims of the study were three-fold:

1. To assess the suitability of techniques for measuring cellular and humoral immune responses in resting and exercised animals under controlled laboratory conditions prior to a large scale study with a live pathogen.
2. To examine the effects of exercise and nutrition on immune function without the confounding effects of disease.
3. To determine whether work stress has an effect on the efficacy of vaccination in sheep as a model for larger ruminants.

5.2 Materials & Methods

5.2.1 The experimental design

A 2 × 2 factorial design was used to examine the immune responses of four groups of sheep to two antigen preparations, using a technique developed by Lloyd and Walker (1993). Two of the groups were exercised daily, five days a week for 19 weeks and two were not exercised. One exercised and one unexercised or rested group were fed on a high plane of nutrition (groups EH and RH respectively), the other two groups were fed on a low plane (EL and RL). Eight weeks after the start of the trial all animals were injected with two antigen preparations, one chosen to stimulate B lymphocytes, the other T lymphocytes. These inoculations were repeated five weeks later. Five days after the booster inoculations the sheep were challenged with a series of five titrated doses of each antigen injected intradermally.

The animals' cellular responses to the intradermal injections were assessed 24 and 48 hrs after challenge using a series of skin measurements at the injection sites. Humoral responses were monitored throughout the experiment by indirect ELISA, measuring antibody titres to the two antigens in sera.

5.2.2 Animals

Twenty-four mature Scottish Blackface ewes of similar age and condition were used for the experiment. They were divided into four groups of six, with an equal number of lighter and heavier animals allocated to each group. All sheep were dosed with fenbendazole (10% w/v) at 1 ml/ 20kg (Panacur SC ® Hoechst Animal Health Ltd, UK) before the experiment and were visually examined by a veterinarian for signs of ill-health. No other clinical history was available.

The four groups were penned in four adjacent pens for the duration of the study. Sawdust was used for bedding. Exercised sheep were run through a footbath containing a 2% formalin solution daily after exercise to keep their feet in good condition, unexercised sheep were footbathed once a week. All animals were weighed once a week using a mechanical weigh crate (Ritchie Farm Equipment Ltd.).

5.2.3 Nutrition

The sheep were fed on hay, those on the high plane of nutrition also received commercial sheep nuts (Seafield Mill Ltd). Maintenance ME requirements of individual animals were calculated using the MAFF (1984) recommendations, based on mean liveweights over a three week period prior to the experiment (n = 3). Groups EH and RH on the high plane of nutrition, were offered hay and concentrate equivalent to approximately 1.5 times their ME requirements for maintenance, EL and RL on the low plane received sufficient hay for maintenance alone. Water was available *ad libitum* at all times except during exercise, when it was offered once every hour during a five minute rest period.

5.2.4 Exercise

Groups EH and EL were exercised as a flock, using a circular horsewalker from which two of the paddles had been removed to provide more space. The sheep were walked round the horsewalker track at a brisk but uniform pace governed by the speed of the paddles, for up to 3 hrs/day, five days a week for 19 weeks. They were rested for five minutes every hour to comply with Home Office animal welfare requirements. The distance the sheep walked each day was monitored using an odometer (Trumeter Ltd.) attached to one of the paddle beams (Plate 5.1). Groups RH and RL remained penned up while the other two groups were exercised.

Initially the sheep were only exercised for 2.5 h/day, but wore weighted belts around their girths to increase energy expenditure. Unfortunately the belts which weighed 10% of the individual animals' pre-experiment mean liveweights, had to be discarded for welfare reasons after 10 weeks when some of the sheep developed skin abrasions. Exercise time was increased by 30 minutes /day to compensate for this and ensure similar levels of energy expenditure by the sheep. Energy expenditure for work was calculated using the factorial method developed by Lawrence (1985) and detailed in section 3.1.3 with the following parameters:

A = energy used to move 1 kg of body weight 1 m horizontally, 2.6 J/m (ARC, 1980)

B = energy used to move 1 kg of applied load 1 m horizontally, 2.6 J/m (Lawrence and Stibbards, 1990)

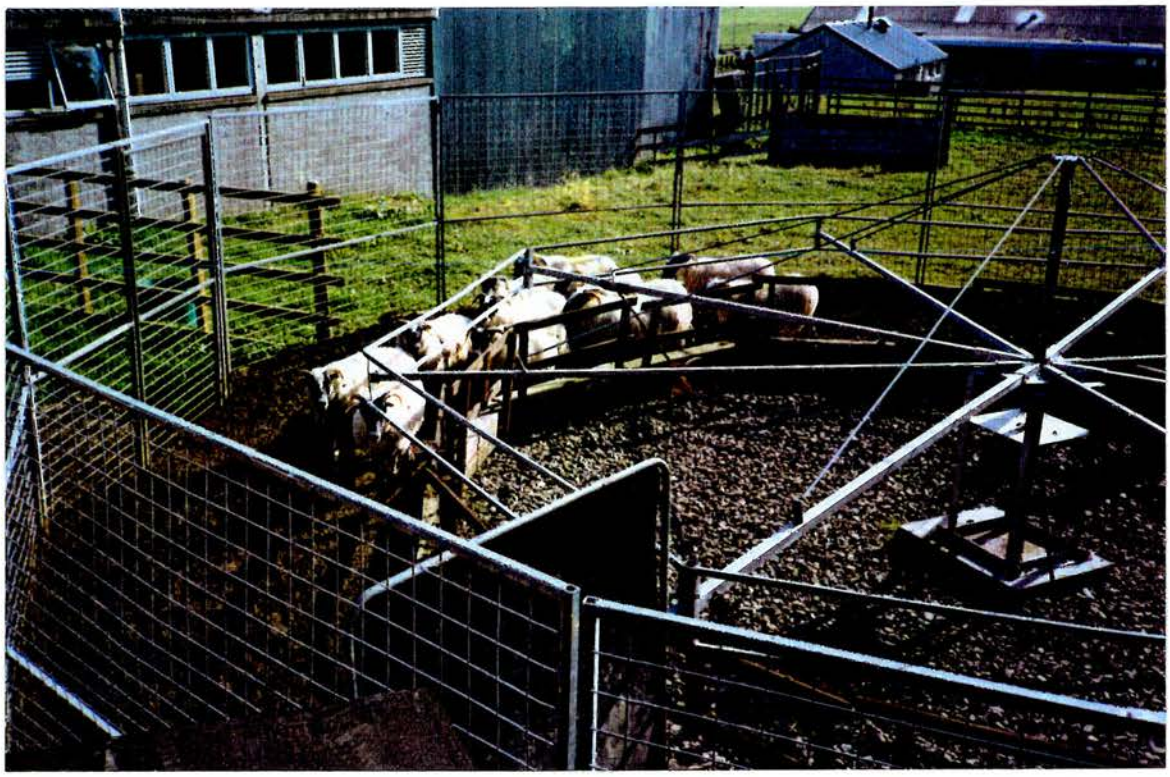


Plate 5.1 : Sheep being exercised, with the odometer attached to the paddle beam visible in the centre of the picture.

5.2.5 Sensitisation with antigens

After eight weeks exercise or rest (day 56) all the sheep were injected with two antigen preparations; (a) Chicken egg ovalbumin (Sigma grade V 98%, Sigma Chemical Co. Ltd.); and (b) *Brucella abortus* (S99) (Central Veterinary Laboratory, Weybridge). *B.abortus* was supplied freeze-dried in 0.5% phenol saline. To remove the phenol it was washed, centrifuged and resuspended three times in distilled water. After the third wash the pellet was resuspended in distilled water and freeze dried.

Sensitisation to each antigen was achieved by intramuscular injection of anhydrous suspensions of each antigen in Freund's incomplete adjuvant using a Dermojet injector (Etablissements Akkra, France). 70 mg of the ovalbumin was ground up with 3.5 ml of adjuvant. The resulting paste was transferred to a glass vial and made up to 7 ml with a further 3.5 ml of adjuvant. A suspension of 70 mg of *B.abortus* prepared in the same manner in 7 ml of adjuvant, was then added to the ovalbumin giving a total volume of 14 ml containing 70 mg of each antigen. The mixture was sonicated for 10 minutes at room temperature before being injected into the sheep.

Each animal received five simultaneous intramuscular injections of 100 µl each, giving a total dose of 2.5 mg of each antigen in 500 µl of adjuvant. Injections were given in the right fore-shoulder which had previously been shaved.

After a further five weeks exercise or rest the sheep were given booster injections of 1.25 mg of each antigen in a total of 500 µl of adjuvant (day 94). The antigens were prepared as before and injected as 5 × 100 µl injections using the Dermojet, this time into the left fore-shoulder. Seven days after the boosters were administered the skin thickness at the site of injection was measured using a pair of internal callipers.

5.2.6 Antigen challenge

Five days after the booster inoculations (day 99) each sheep was challenged by intradermal injection of five titrated doses of each antigen in sterile phosphate buffered saline (PBS) (Table 5.1). 100 µl doses of the antigens were injected into 10 cm squares marked on the rumps of the animals, one dilution per square, ovalbumin into the left rump, *B.abortus* into the right. *B.abortus* being insoluble in water had to be sonicated for 10 minutes on ice to produce a suspension suitable for injection. The injections were given intradermally into the centre of each square using plastic disposable syringes with 26 gauge needles.

Ovalbumin (µg / 100 µl of PBS)	***B.abortus* (µg / 100 µl of PBS)**
2500	1250.0
500	250.0
100	50.0
20	10.0
4	2.5

Table 5.1 : The dilution series used for the antigen challenges.

5.2.7 Skin test responses to the antigenic challenges

Skin test reactions were assessed at each of the five injection sites for each antigen, 24 and 48 hours after challenge. The skin fold thickness and diameter of any visible reaction site were measured using a pair of internal callipers (Plate 5.2) and the response was quantified as the product of the two measurements. For analysis the animal group median response to each dose of antigen was calculated from the individual responses after 24 and 48 hours.

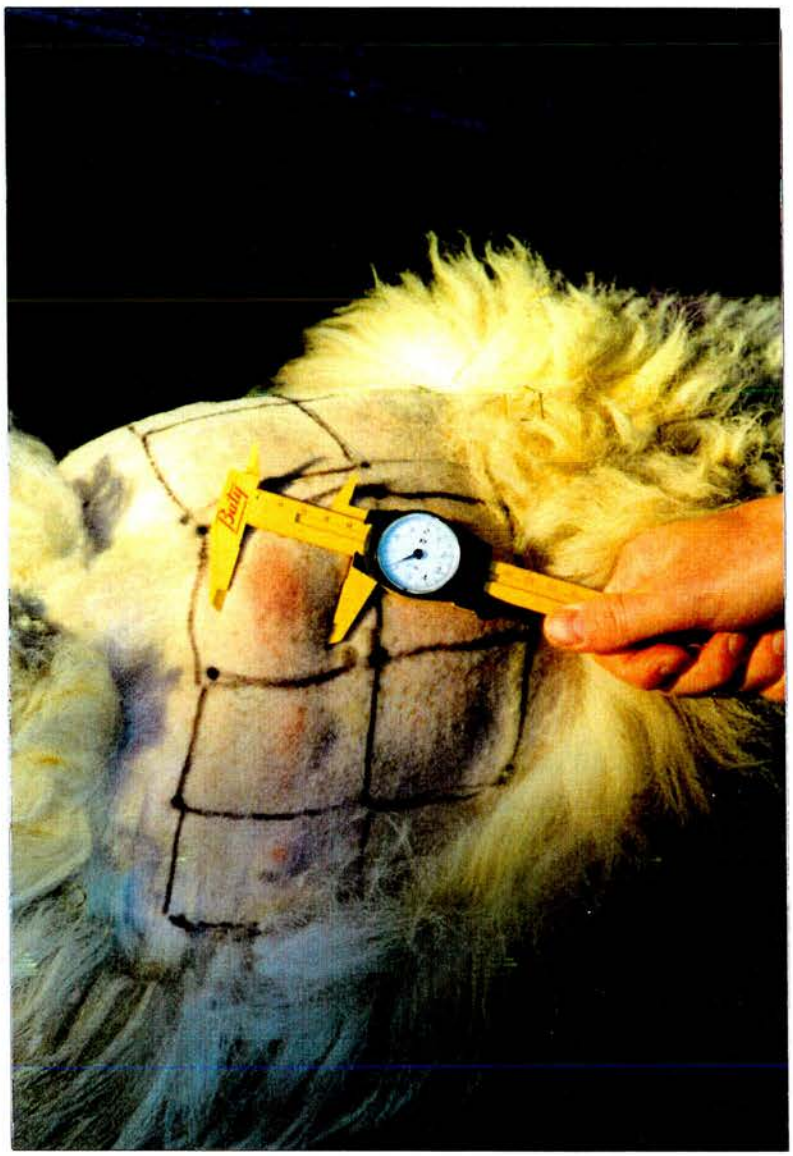


Plate 5.2 : Measuring the diameter of one of the antigen injection sites on the rump of one of the sheep 48 hours after challenge.

5.2.8 Blood sampling

All sheep were blood sampled once a week for 8 weeks until their first antigen injections and thereafter twice a week. 5 ml samples were collected from the jugular vein of each animal into plain glass vacutainers. The blood was allowed to clot and the serum extracted for ELISA. Two 800 µl aliquots of sera were harvested from each vacutainer and stored in sterile microtubes at –20°C until required.

5.2.9 ELISA's to measure antibody responses

Two indirect antibody ELISA's were used to measure sheep antibody responses to the activating antigens, with seven parameters being employed to characterise the responses of the sheep to each antigen:

1. The magnitude of the response 15 days after the first injection.
2. The magnitude of the response 29 days after the first injection.
3. The magnitude of the peak antibody response to the first injection (peak primary response).
4. The time lag between the first injection and the peak response.
5. The magnitude of the response 15 days after the booster injection.
6. The magnitude of the peak antibody response to the booster injection (peak secondary response).
7. The time lag between the booster injection and the peak response.

The ELISA's were performed according to the protocol detailed in section 3.2.1, specific details are given below.

Preparation of antigens for ELISA

Ovalbumin

A stock solution of ovalbumin was made up in distilled water at a concentration of 1 mg/ml of water in sufficient quantity for all the ELISA's. The solution was aliquoted into 120 µl volumes, sufficient for two ELISA plates and stored at –20°C until required.

B. abortus

The *B. abortus* bacterial cells were suspended in PBS and washed three times by repeated centrifugation at 4000 rpm for 15 minutes. Between washes the bacterial pellet was resuspended in fresh PBS. After the third wash PBS was replaced by TRIS - HCL buffer at pH 8.5. An equal volume of small glass beads (ballotini, Grade 14, Jencons Scientific Ltd.) was added to the suspension and the mixture was sonicated on ice at maximum power for 20 minutes. After centrifugation for 15 minutes at 2500g the supernatant antigen preparation was drawn off and stored at –20°C.

ELISA protocols

Appropriate dilutions for the various components of each assay were established using series of chequer-board titrations as outlined in section 3.2.3. The working dilutions used for each assay are given in Table 5.2. A donkey anti-sheep IgG (whole molecule) peroxidase conjugated antiglobulin preparation (Sigma Chemical Co. Ltd.) was used in both assays.

ELISA	Ovalbumin	*B. abortus*
Antigen used	Ovalbumin	*B. abortus*
Antigen dilution	1:800	1:1600
Serum dilution	1:1000	1:8000
Conjugate dilution	1:5000	1:5000

Table 5.2 : Dilutions used for the components of the two ELISA's

The consistency of each assay was monitored with six wells of positive and six wells of negative control serum run on every plate and their means used for quality control. The positive control serum had been collected from a sheep 35 days after it had received booster injections of ovalbumin and *B. abortus*, the negative control came from an animal which had not been exposed to the two antigens (both were supplied by Dr C.M. Lloyd, CTVM).

Correction of inter-plate variation using the F factor

All optical density (OD) results were corrected for plate to plate variation using a calibration factor (F) derived from the OD of the plate positive and negative controls (Luckins, 1983) based on correcting all plates back to a single plate judged to be representative of the assay, designated "plate 0". In this study the plate which had positive and negative control OD's nearest to the mean positive and negative OD's for all plates was selected as "plate 0". The F factor for individual plates was then calculated as follows:

Where,

$$F = \frac{P_0 - N_0}{P_t - N_t} \quad \textbf{Equation 5.3}$$

P_0 = OD of the positive control serum on plate 0

N_0 = OD of the negative control serum on plate 0

P_t = OD of the positive control serum on the plate under test

N_t = OD of the negative control serum on the plate under test

The F values calculated for each plate in each assay are given in appendices 3 and 4. Each OD for each test serum on the plate was then adjusted thus:

$$\textbf{Corrected OD} = F \times (OD_{serum} - N_t) + N_0 \quad \textbf{Equation 5.4}$$

5.2.10 Data analysis

1. A two-way ANOVA was used to determine the significance of group differences in animal liveweights at the start and at the end of the trial, attributable to exercise and plane of nutrition.

2. The median skin responses of each of the four groups of sheep to each antigen dose were compared using Friedman's tests (Siegal and Castellan, 1988). One test was used to compare responses to ovalbumin another for responses to *B. abortus*.

3. Mann-Whitney tests were used to examine differences in skin responses and in antibody responses caused by plane of nutrition and exercise.

4. Kruskal Wallis tests were employed to test the significance of group differences in skin thickness measurements taken after the booster sensitisation, also to test group differences in antibody response to each of the two antigens, using the seven parameters detailed in section 5.2.9.

5. Peak antibody responses to the two antigens were tested using the least squares method of linear regression to see if they were correlated. Primary and secondary peak responses were compared separately.

5.3 Results

Exercise at the intensity and duration imposed on the sheep in this study had no significant effect on either humoral or cellular responses to the two antigens as measured by ELISA and skin testing respectively. Similarly the two planes of nutrition used did not significantly affect responses to either of the two antigens, with the exception of the primary humoral response to ovalbumin which was significantly faster in the sheep on the high plane of nutrition than in those on the low plane. Both plane of nutrition and exercise did however significantly affect animal liveweight changes over the experiment. Exercised sheep lost weight whilst their rested contemporaries gained weight and similarly animals on the low plane of nutrition lost weight whereas those on the high plane gained.

5.3.1 Animal liveweight changes

Exercise and low nutrition caused significant liveweight losses over the study (Table 5.3). Exercised sheep lost a mean of 11 ± 6.2 g/day over the study whilst their rested contemporaries gained 12 ± 6.2 g/day (n = 12). Similarly sheep on the low plane of nutrition lost an average of 16 ± 4.8 g/day whereas those on the high plane gained 17 ± 5.3 g/day (n = 12).

There were also significant differences in the liveweight changes of the four groups of sheep over the study (Table 5.3). Exercised animals fed on the low plane of nutrition (group EL) lost weight whereas sheep which were rested and fed on the high plane (RH) gained weight. The liveweights of the animals in the other two groups remained almost static. By the end of the study the animals in EL had lost an average of 3.6 ± 0.57 kg while those in RH had gained 3.9 ± 0.52 kg (n = 6). The sheep in EH gained only 0.7 ± 0.93 kg, those in RL lost a similar amount, 0.7 ± 0.81 kg

(n = 6). The mean liveweights of the four groups at the start of the experiment were not significantly different.

Group	Initial LW (kg)		Liveweight change (g/day)	
	mean	s.e.	mean	s.e.
RL	55.9	3.35	− 5	6.1
EL	57.5	1.68	− 27	4.3
RH	58.8	3.46	29	3.9
EH	58.0	1.95	5	6.9
ANOVA	**F**	**P**	**F**	**P**
Group	0.19	0.90 ns	18.27	<0.001 ***
LSD			16.0	
Exercise	0.02	0.88	17.94	<0.001 ***
Nutrition	0.37	0.55	36.82	<0.001 ***
Ex. × Nutr.	0.18	0.67	0.05	0.82 ns

Table 5.3 : Group mean liveweights (kg) at the start of the experiment and liveweight changes (g/day) over the experiment (n = 6), with ANOVA results.

5.3.2 Distances walked and energy expended

The mean distance walked by the exercised sheep each day on the horsewalker was 5929 ± 147.4 m (n = 47) when wearing weight belts, which increased to 8637 ± 166.0 m (n = 31) once the belts were removed and the exercise time was extended by 30 minutes. The average speeds were 0.66 ± 0.017 ms^{-1} with belts, 0.82 ± 0.014 ms^{-1} without. The mean estimated ME expenditures for work with and without belts were 17 ± 0.1% and 23 ± 0.1% of the animals ME requirements for maintenance respectively.

5.3.3 Skin tests

All the sheep showed marked skin reactions to both antigenic challenges ovalbumin and *B. abortus*, with reddening and swelling of the skin around injection sites. The magnitude of the response (quantified as the skin fold thickness multiplied by the diameter of any visible reaction at the injection site) was related to the antigen dose applied, the largest doses eliciting the biggest responses. There were however no significant differences in the median skin responses of the four groups of sheep to ovalbumin by Friedman's test ($S = 5.4$, $P = 0.15$, $n = 5$). Similarly there were no significant differences in the responses of the groups to *B. abortus* ($S = 2.5$, $P = 0.47$, $n = 5$). The median reaction of each group of sheep to each antigen dose is given in Table 5.4 and Table 5.5.

Reactions to specific doses of antigen varied considerably between animals within groups as is evident from the large semi interquartile ranges (s.i.r.) in the tables. Not all of the animals produced a visible response to all 5 intradermal injections of each antigen, however there was no obvious pattern to these failures.

Skin responses to the two antigens did not appear to be affected either by plane of nutrition or by exercise. There were no significant differences in the responses of the animals on the high and low planes of nutrition to either ovalbumin or *B. abortus,* by Mann-Whitney test ($W = 94$, $P = 0.43$ and $W = 102$, $P = 0.85$ respectively, $n = 10$). Similarly there were no significant differences in the responses of the exercised and rested sheep to either ovalbumin or *B. abortus* ($W = 111$, $P = 0.68$, $n = 10$ for both antigens).

Antigen dose	Skin reactions (Skin fold thickness × diameter) (mm²)							
	EH		RH		RL		EL	
(μg/100μl)	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.
2500	544	190.0	974	494.5	571	261.8	461	343.4
500	367	302.0	619	453.5	238	238.3	241	110.3
100	124	58.5	118	44.0	180	61.4	100	16.8
20	96	31.6	88	57.2	81	60.9	59	8.6
4	67	42.5	93	37.5	52	58.0	68	18.5

Table 5.4 : Group median skin reactions to ovalbumin at each injection site.

Antigen dose	Skin reactions (Skin fold thickness × diameter) (mm²)							
	EH		RH		RL		EL	
(μg/100μl)	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.
1250	756	173.5	867	502.5	739	114.0	791	262.5
250	381	187.3	461	241.0	388	170.3	359	189.5
50	148	140.4	178	125.4	230	98.2	179	82.1
10	191	59.9	177	57.6	162	55.3	86	28.2
2	110	43.9	73	28.8	120	77.9	62	30.7

Table 5.5 : Group median skin reactions to *B.abortus* at each injection site.

5.3.4 Skin responses to booster injections

The booster sensitisation injections elicited similar skin responses from the animals to those seen during the skin tests. There were no significant group differences in skin fold thickness at the injection sites one week after injection, by Kruskal Wallis test ($H = 2.8$, $P = 0.42$, $n = 30$), furthermore neither plane of nutrition nor exercise had a significant effect on the responses (Mann-Whitney tests: $W = 3675$, $P = 0.81$ and $W = 3852$, $P = 0.25$ respectively, $n = 60$).

5.3.5 Antibody responses

Neither plane of nutrition nor exercise affected the pattern and magnitude of the serum antibody responses of the sheep to each antigen, measured by ELISA, with one exception (Table 5.6 and Table 5.7). The interval between the primary injection of ovalbumin and the peak response was significantly longer in animals on the low plane of nutrition, 27 days compared to 22 days in the animals on the high plane.

The antibody responses of all the sheep to the two antigens showed a similar pattern, characterised by a small initial increase after the primary injection which reached a peak 18 - 29 days after the injection. This was followed by a much larger and more rapid increase after the booster which peaked within 12 - 19 days (Figure 5.1 and Figure 5.2). Initial responses however varied considerably between individual animals within the groups. Some sheep produced large amounts of antibody after the primary injection, others produced hardly any. In some animals antibody titres declined 4 to 5 weeks after the injection, in others they were still increasing when the booster injection was administered. Responses to the booster were more uniform and more persistent than the primary responses. At the end of the study, 8 weeks after the booster injections, the serum antibody levels of all animals to both antigens remained at or above the peak responses elicited by the primary injections.

There were no significant differences in the peak antibody responses of the four groups to the primary injection of ovalbumin, nor in the time interval between injection and peak response; similarly for the booster injection (Table 5.8). There were also no significant group differences in the peak responses to *B. abortus* nor in the time lags between injection and peak response (Table 5.9). The similarities in the antibody responses of each group were confirmed by comparing the magnitude of the

Sheep group	Peak response to the primary injection (ELISA OD)		Interval between primary injection and peak response (days)		Peak response to the booster injection (ELISA OD)		Interval between booster and peak response (days)		Antibody response 15 days after primary injection (ELISA OD)		Antibody response 29 days after primary injection (ELISA OD)		Antibody response 15 days after booster injection (ELISA OD)	
	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.
Rest	0.477	0.2420	22	6.5	1.377	0.1011	15	3.0	0.377	0.2578	0.289	0.2592	1.377	0.0807
Exercise	0.453	0.1654	22	4.0	1.432	0.0885	14	3.5	0.341	0.1187	0.373	0.1370	1.353	0.0694
$W_{exercise}$	50		133		157		150		160		145		157	
$P_{exercise}$	1.00 ns		0.31 ns		0.73 ns		1.00 ns		0.60 ns		0.77 ns		0.71 ns	
Low	0.550	0.2236	27	4.6	1.377	0.0934	14	1.5	0.364	0.2340	0.388	0.2720	1.353	0.0738
High	0.452	0.1259	22	2.7	1.446	0.0812	17	3.5	0.354	0.1492	0.274	0.1027	1.353	0.0808
$W_{nutrition}$	163		185		132		127		161		165		137	
$P_{nutrition}$	0.48 ns		0.03 *		0.30 ns		0.17 ns		0.56 ns		0.42 ns		0.47 ns	

Table 5.6 : Characteristics of the antibody responses of exercised and rested sheep, and of sheep on high and low planes of nutrition to ovalbumin injections, with Mann-Whitney test results (n = 12).

Sheep group	Peak response to the primary injection (ELISA OD)		Interval between primary injection and peak response (days)		Peak response to the booster injection (ELISA OD)		Interval between booster and peak response (days)		Antibody response 15 days after primary injection (ELISA OD)		Antibody response 29 days after primary injection (ELISA OD)		Antibody response 15 days after booster injection (ELISA OD)	
	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.
Rest	0.829	0.1422	25	6.1	1.472	0.0403	19	6.1	0.669	0.2200	0.751	0.1764	1.420	0.0600
Exercise	0.822	0.1224	22	4.1	1.468	0.0768	15	5.6	0.606	0.1697	0.576	0.1569	1.428	0.0831
$W_{exercise}$	154		162		165		165		153		167		158	
$P_{exercise}$	0.84 ns		0.51 ns		0.42 ns		0.39 ns		0.89 ns		0.34 ns		0.67 ns	
Low	0.807	0.1829	25	6.1	1.472	0.0389	14	4.6	0.589	0.2050	0.592	0.2276	1.441	0.0497
High	0.834	0.1136	22	7.3	1.468	0.0397	17	5.5	0.652	0.1558	0.646	0.1600	1.415	0.0723
$W_{nutrition}$	154		170		154		129		138		155		168	
$P_{nutrition}$	0.84 ns		0.25 ns		0.86 ns		0.23 ns		0.51 ns		0.80 ns		0.31 ns	

Table 5.7 : Characteristics of the antibody responses of exercised and rested sheep, and of animals on high and low planes of nutrition to *B. abortus* injections, with Mann-Whitney test results (n = 12).

responses at fixed intervals after each injection, namely 15 and 27 days after the primary injection and 15 days after the booster. Again there were no significant group differences in the size of the responses to each of the antigens on each occasion (Table 5.8 and Table 5.9).

There were significant positive linear correlations between peak primary responses to the two antigens regardless of exercise or plane of nutrition ($R \geq 0.60$, $P \leq 0.04$, $n = 12$). Animals that produced large amounts of antibody to one antigen also produced large quantities of antibody to the other. There were however no significant correlations between secondary responses to the two antigens ($R \leq 0.39$, $P \geq 0.21$, $n = 24$). The antibody responses of each animal to ovalbumin and *B. abortus* measured by ELISA are given in appendices 26 and 27 respectively.

Figure 5.1 : Median ovalbumin antibody OD values for each group measured by ELISA.

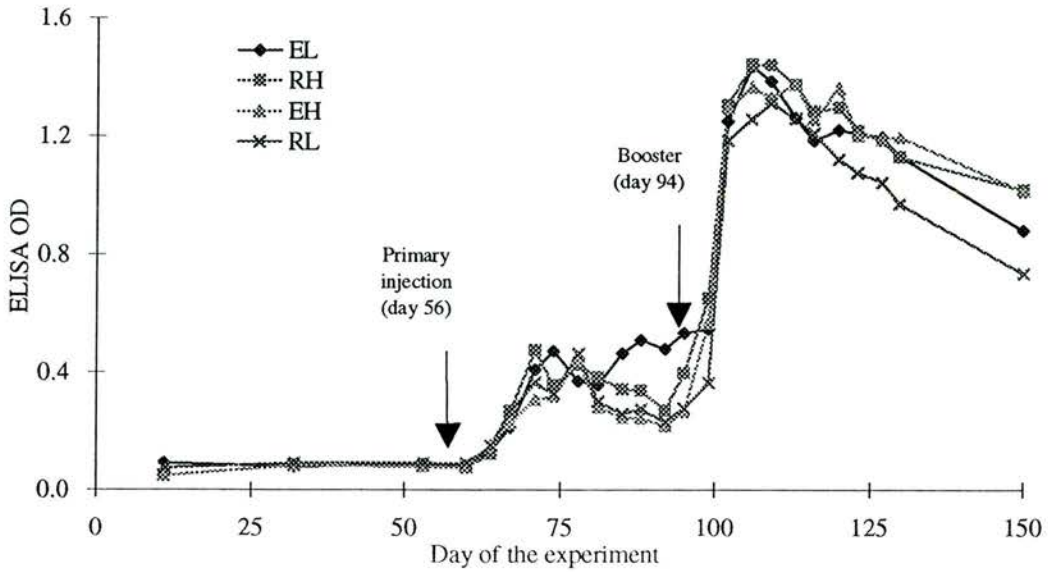
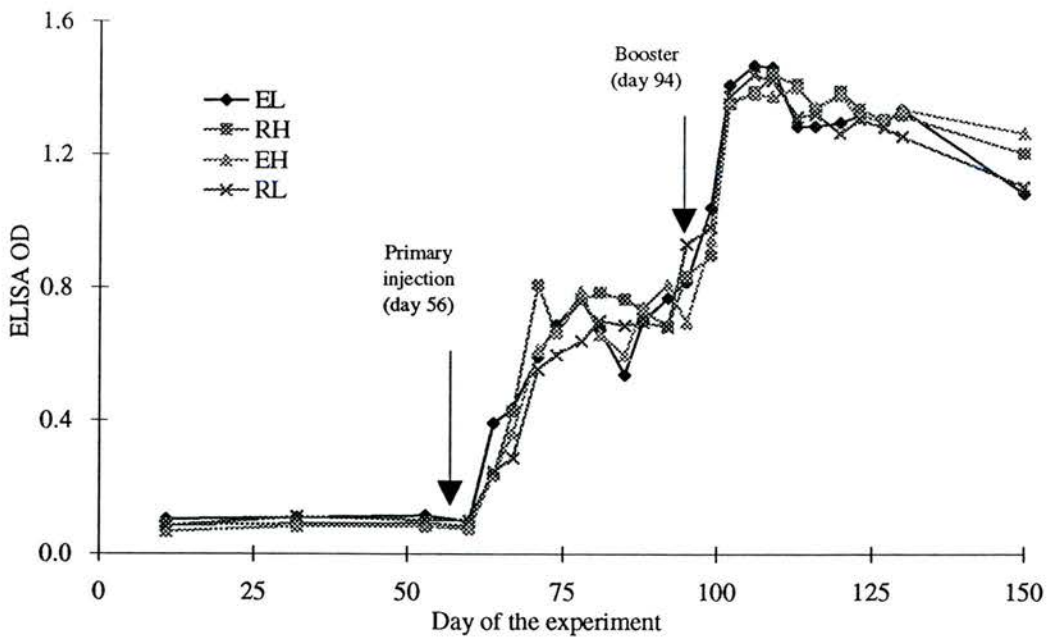


Figure 5.2 : Median *B.abortus* antibody OD values for each group, measured by ELISA.



Sheep group	Peak response to the primary injection (ELISA OD)		Interval between primary injection and peak response (days)		Peak response to the booster injection (ELISA OD)		Interval between booster and peak response (days)		Antibody response 15 days after primary injection (ELISA OD)		Antibody response 29 days after primary injection (ELISA OD)		Antibody response 15 days after booster injection (ELISA OD)	
	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.
EH	0.439	0.0954	22	0.0	1.432	0.0737	17	4.0	0.307	0.1622	0.245	0.0794	1.328	0.0527
RH	0.518	0.1895	19	5.3	1.475	0.1121	17	3.5	0.475	0.2305	0.341	0.1860	1.438	0.0876
RL	0.463	0.3385	24	4.8	1.332	0.0823	15	1.5	0.363	0.3455	0.259	0.3495	1.312	0.1479
EL	0.616	0.2132	29	5.5	1.441	0.1271	12	3.5	0.409	0.1920	0.464	0.1629	1.384	0.1217
H	1.38		5.95		2.03		2.03		1.63		1.66		2.49	
P_{group}	0.71 ns		0.12 ns		0.57 ns		0.61 ns		0.65 ns		0.65 ns		0.48 ns	

Table 5.8 : Characteristics of the antibody responses of each group of sheep to ovalbumin injections, with KW test results (n = 6).

Sheep group	Peak response to the primary injection (ELISA OD)		Interval between primary injection and peak response (days)		Peak response to the booster injection (ELISA OD)		Interval between booster and peak response (days)		Antibody response 15 days after primary injection (ELISA OD)		Antibody response 29 days after primary injection (ELISA OD)		Antibody response 15 days after booster injection (ELISA OD)	
	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.	median	s.i.r.
EH	0.819	0.1044	22	7.3	1.437	0.1230	15	7.1	0.610	0.1043	0.595	0.1544	1.373	0.0986
RH	0.834	0.1638	22	7.9	1.474	0.0237	19	7.3	0.805	0.2725	0.763	0.1785	1.438	0.0707
RL	0.807	0.1651	27	5.9	1.460	0.0726	17	5.9	0.551	0.1769	0.685	0.2168	1.420	0.0725
EL	0.839	0.2725	24	3.8	1.474	0.0510	12	2.9	0.589	0.3310	0.536	0.2585	1.460	0.0599
H	0.32		2.83		1.40		2.74		1.26		1.17		2.37	
P_{group}	0.96	ns	0.42	ns	0.71	ns	0.43	ns	0.74	ns	0.76	ns	0.50	ns

Table 5.9 : Characteristics of the antibody responses of each group of sheep to *B. abortus* injections, with KW test results (n = 6).

5.4 Discussion

The results from this experiment suggest that undernutrition may depress sheep humoral responses to ovalbumin, but that it has no effect on humoral responses to *B. abortus*, nor any effect on cellular responses to either antigen. Exercise at the intensity used here had no significant effect on the cellular or humoral immune responses of sheep, measured by skin tests and ELISA respectively. It is possible however that the exercise stress imposed on the animals was not severe enough to affect the immune responses measured, or alternatively that the techniques used to measure responses may not have been sufficiently sensitive to detect any changes which did occur.

Nathan *et al* (1977) reported similar variations in humoral responses to different antigens in underfed poultry, to those observed in this experiment. Starvation has been shown to enhance antibody responses to Newcastle disease vaccination (Spalatin and Hanson, 1974) but depress antibody responses to both *E. coli* and sheep erythrocytes (Nathan *et al*, 1977) in chickens. Starvation at the time of vaccination did not however affect the subsequent survival of the birds when challenged with virulent Newcastle disease virus (Spalatin and Hanson, 1974). Kelley (1980) proposed that the differences in the poultry antibody responses to starvation may have been due to differences in the fasting times coupled with differences in the antigens used.

Undernutrition is normally associated with a depression in cell mediated immune responses (Tizard, 1992) however the degree of undernutrition used in this experiment may not have been severe enough to depress cell mediated responses to either of the antigens used. In those experiments that have showed depressed cell mediated responses the degree of undernutrition has been much greater than in this study. Amkraut, Solomon, Kasper and Purdue (1973) demonstrated that reducing the

food intake of rats to only 50% of *ad libitum* intake severely depressed their cell mediated responses. Similarly semi-starvation in humans is known to reduce circulating lymphocyte numbers (Bistrian *et al*, 1975). In this study however even the sheep in group EL, the most underfed animals, received sufficient feed to meet 80% of their total energy requirements for maintenance and exercise. A greater degree of starvation might have significantly depressed cell mediated immune responses to one or other antigen, it might also have further depressed humoral response to ovalbumin, however it would not have been acceptable on animal welfare grounds given the long duration of the study.

The relatively modest weight loss suffered by the sheep in EL over the course of the experiment, an average of 6.3% of initial body weight, confirmed that the degree of undernutrition imposed on the animals was mild. Weight losses of draught animals in the tropics can be much more pronounced, particularly over the dry season in sub-Saharan Africa. Wilson (1987) reported that in the semi-arid zone of Mali, draught oxen can lose up to 25% of their liveweight during the dry season. Weight losses of that magnitude although they might have suppressed immune responses, would not have been acceptable in this study.

The exercise imposed on groups EL and EH in this experiment, was not particularly arduous for reasons of animal welfare. The sheep walked an average of 5.9 km/day when wearing the weight belts, 8.6 km once they were removed. In comparison sheep kept in a 0.4 Ha paddock have been found to walk up to 5.3 km/day whilst grazing (Tribe, 1949) and extensively grazed sheep in Australia can walk as much as 14 km/day (Squires, Wilson and Daws, 1972). The AFRC (1993) recommendations on the energy requirements of hill sheep in the UK such as the blackfaces used in this study, assume that they walk an average of 5 km per day and climb 200 m. For a 50 kg sheep such activity would require an energy expenditure of approximately $1.19 \times ME_{maint}$. The mean estimated daily energy expenditure of the exercised sheep in the experiment was very similar to this, ranging from $1.17 \times$

ME_{maint} when the animals were wearing weight belts to 1.23 × ME_{maint} without the belts. The only difference being that in the study the exercise was performed in one short intense period, whereas in the field sheep normally walk only a few paces at a time before stopping to eat. Typical energy expenditures for working draught animals on farms tend to be higher, ranging from around 1.25 to 2 × ME_{maint} (Alford, 1994; Lawrence, 1985; Pearson *et al*, 1989) depending upon the task being performed and the duration of the work. Goe and McDowell (1980) suggested they may occasionally reach 2.7 × ME_{maint}.

To increase the energy expenditure of a 50 kg sheep in this study to even 1.5 × ME_{maint} would have meant walking it a distance of approximately 19 km/day, or 15 km if the animal had carried a weight equal to 25% of its body weight. At the speeds recorded here this would have taken between 6.5 and 7 h/day which was impractical. The weight belts which were used were equivalent to only 10% of animal liveweight. Energy expenditures for work could also have been increased by walking the animals up a gradient (Lawrence, 1985) or by exercising the animals in deep mud (Dijkman, 1993) or both.

In other studies the effects of exercise on immune responses appear to vary markedly with the intensity and duration of exercise undertaken. Chronic exercise involving running for 1 h/day for 10 weeks, has been shown to reduce T cell activity in rats whilst a single bout of acute exercise at up to 75% of the animals' maximal oxygen uptake enhanced T cell responses (Lin and Chen, 1993). In the same experiment both chronic and acute exercise enhanced B cell responses. A number of other studies have demonstrated that forced running or swimming for long periods or at high intensity can inhibit tumor growth in rats (Baracos, 1989; Deuster, Morrison and Ahrens, 1985; Hoffman, Paschkis, DeBias, Cantarow and Williams, 1962; Rusch and Kline, 1944). Running also appears to increase antibody production in mice. Work by Liu and Wang (1987) has shown that antibody titres to *Salmonella typhi* in mice running for 10 minutes twice a day were on average 2.76 times higher than in the

resting controls up to 13 weeks post immunisation. In contrast Anderson *et al* (1991) found that only 7 minutes exercise on a treadmill running at 7 ms^{-1} on an incline of 3° was sufficient to make cattle more susceptible to experimental pasteurellosis, exercise having apparently delayed the egress of neutrophils from the blood to the lung alveoli; important in combating the disease. In horses a single bout of exhaustive exercise lasting no more than 11.5 minutes including a 6 minute warm up phase transiently impaired neutrophil antimicrobial functions although it had no effect on immunoglobulin levels nor on lymphocyte function (Wong *et al*, 1992). As already indicated, working the sheep harder in this experiment would have had serious animal welfare implications.

Although only one significant difference was seen in all the cellular and humoral parameters measured in this study, it seems unlikely that the techniques used to measure responses were not sufficiently sensitive to detect changes that did occur as a result of exercise or undernutrition. Both antigens proved effective in eliciting strong cellular and humoral responses from the sheep, ovalbumin having been selected as a T-lymphocyte activator, *B. abortus* as a B-lymphocyte activator (Roitt, 1980b). The ELISA's showed clear primary and secondary responses to sensitisation and the skin tests indicated a gradation in the cellular responses depending upon the concentration of the antigen injected, although there was considerable variation in both the cellular and humoral responses of individual animals within each of the four groups. The large variations in the primary antibody responses of sheep within the same group to each of the antigens were probably due to differences in individual responsiveness rather than to prior exposure to either antigen. It is extremely unlikely that any of the animals had previously been in contact with either ovalbumin or *B. abortus*.

The antigens and the techniques used in this study were selected on the basis that they had previously been successfully employed by Lloyd and Walker (1993) to demonstrate that adult *A. variegatum* ticks caused immunosuppression in sheep

infected with *Dermatophilus congolensis*. It is however possible that the immunosuppressive mechanisms employed by adult ticks differ from those involved in exercise or nutrition induced immunosuppression.

In their experiment Lloyd and Walker (1993) found significant group differences in the skin tests responses and in the antibody responses by ELISA of three groups of four sheep to both the antigens used in this study. One group of animals infested with adult *A. variegatum* ticks showed reduced skin test responses to both ovalbumin and *B. abortus* compared to the responses of a group infested with nymphs of the same tick species and a control group without ticks. Similar differences were observed in skin thickness at the site of the booster antigen injections. Antibody responses to the two antigens also followed the same pattern and were significantly weaker in the sheep infested with adult ticks.

Following the success of their experiment Lloyd (1993) concluded that skin tests were a very useful technique for measuring cellular immune responses in the field. In this study however the skin responses of some animals varied considerably between injections; several concentrations of an antigen producing pronounced tissue swelling and thickening, whilst other stronger concentrations produced no visible reaction on the same individual. This inconsistency in the cellular response might be ascribed to inconsistent technique when injecting the antigens. Intradermal injections are difficult to administer consistently and here 240 of them had to be given in a single session. In contrast Lloyd and Walker (1993) carried out their experiment in two separate phases with three groups of two animals in each phase, administering only 60 intradermal injections at a time which probably improved the consistency. It is also possible that the injection sites used in this study might have reduced the sensitivity of the assays, although this seems unlikely as Lloyd and Walker (1993) used the same site. Skin tests are normally performed in the sensitive neck or anal fold tissue (Tizard, 1992). In this instance the rump had to be used because of the large number of injections being given.

Griffin (1989) recognised that when skin tests are used to quantify cell mediated immune responses it can be difficult to assess the results accurately, however he stated that they remain the only practical way of monitoring such responses *in vivo*. Furthermore skin tests have been successfully used to demonstrate the effects of stress on cell mediated immunity in a range of animal species including; mice (Blecha *et al*, 1982; Pitkin, 1965); cattle (Kelley *et al*, 1982); pigs (Westly and Kelley, 1984); and chickens (Regnier and Kelley, 1981). Responses appeared to vary however with the stressor, the timing and duration of the stress with respect to administration of the immunogen and the type of test used to monitor the reaction (Blecha *et al*, 1982; Kelley *et al*, 1982).

Skin tests have particular merits over other techniques when measuring gross differences in cellular immune responses in the field, as results are easily read with the minimum of training and without recourse to expensive equipment or a sophisticated laboratory. *In vitro* techniques based on the use of T-cell activators such as phytohaemaglutinin, concanavalin A or pokeweed mitogen to stimulate lymphokine production, or the use of conjugated monoclonal antibodies and fluorescent-activated cell sorters (FACS) do provide a more accurate assessment of cell mediated responses, but they require the resources of a well-equipped laboratory (Tizard, 1992). This experiment has however demonstrated that without a semi-automated intradermal injector of some description, quantitative skin tests involving multiple injections are inappropriate for large numbers of animals which cannot easily be restrained.

Competition between the two simultaneously injected antigens for MHC (major histocompatibility complex) class II positive macrophages which present antigen to the helper T-cells thereby stimulating an immune response can be discounted as a possible explanation for the absence of group differences in humoral responses in this study; Lloyd and Walker (1993) injected their antigens simultaneously when showing that adult ticks depressed humoral immune responses.

Furthermore in this experiment there were significant positive correlations between primary antibody responses to the two antigens with each group of sheep, suggesting that the animals responded to each antigen independently.

Sheep were used in this study as a model to give pointers to likely immune responses in larger ruminants, such as cattle and buffalo used to provide draught power on farms. Six animals was considered to be the minimum group size necessary for the experiment, although Lloyd and Walker (1993) demonstrated significant differences in immune responses using only four animals per group. Financial and logistical constraints meant that using four groups of six large ruminants was not a viable option for the study. Suitable facilities were not available to exercise 12 cattle simultaneously under controlled conditions, ensuring that each did a similar amount of work, furthermore the labour required for such an undertaking made it prohibitively expensive.

Other scientists have used laboratory rodents to examine the effects of exercise on immunity (Good and Fernandes, 1981; Liu and Wang, 1987), however there are drawbacks to this approach. Fitzgerald (1988) pointed out that laboratory mice are almost certainly chronically under-exercised compared to their wild cousins, so that results purporting to show that running exercise enhances immune responses in mice, may in fact be demonstrating that being unfit depresses immune responses which are subsequently improved by exercise. Sheep and in particular extensively grazed hill breeds such as the blackfaces used in this study, are at least accustomed to walking quite considerable distances each day in search of food (Arnold and Dudzinski, 1978), hence they were considered to be an acceptable substitute for draught animals in this study.

In conclusion, moderate exercise appears to have no effect on the immune responses of sheep to non-pathogenic antigens, suggesting that vaccinating animals under such conditions should have no effect on the protective immunity subsequently achieved. This is of particular importance to the owners of draught animals that may

need to be vaccinated during the working season. Undernutrition did reduce the speed of the primary antibody response to ovalbumin, however it had no effect on any of the other responses measured. Secondly neither exercise nor undernutrition as used in this study, appeared to affect the results of skin tests. If skin tests had been affected by either factor this could cause problems in disease eradication programmes using such tests to screen animals for evidence of infection. For example in tuberculosis eradication programmes in Africa and elsewhere cattle may have to be trekked over long distances to central testing points. Finally, although no marked differences were observed in the immune responses of the four groups of sheep used in this study, other research indicates that different degrees of exercise and nutritional stress may have significant effects on the immune responses of larger ruminants when they are challenged with live pathogens.

6. THE EFFECT OF WORK STRESS ON THE TRYPANOTOLERANCE OF N'DAMA CATTLE ON TWO PLANES OF NUTRITION IN THE GAMBIA

6.1 Introduction

Trypanosomosis is the most important disease constraint on livestock productivity across approximately 10 million square kilometres of sub-Saharan Africa (one third of the continent), threatening some 60 million cattle in 36 countries (Chadega, 1994; FAO, 1994). The disease precludes or seriously restricts the use of animal traction as an alternative to manual labour for small farmers in these countries. Treatment and prophylactic protection of susceptible livestock using drugs, has proved expensive and is often ineffective and unsustainable. Controlling the insect vector across such a large area is impractical. The disease is currently estimated to cost Africa in excess of US $500 million *p.a.* in lost production and control costs (ILRAD, 1993). Exploiting the natural tolerance of some breeds of livestock to the disease offers a practical, sustainable option for increasing livestock productivity in areas where the disease is endemic, however little is known about the interactions of work, nutrition and trypanotolerance in draught animals.

Trypanotolerance is genetically determined (Murray and Trail, 1983), although the degree to which it is manifested in individuals is affected by physiological, environmental and nutritional factors (Murray *et al*, 1982). There are conflicting theories on the effects of work on trypanotolerance. Foy (1911) and Traverse (1972) suggested tolerance can break down when animals are worked, however the evidence

for this is largely anecdotal. Murray and Trail (1983) stated that tolerance is reduced by a number stressors such as pregnancy, lactation, intercurrent disease and malnutrition, as well as work. In contrast results obtained by Ravindran (1988) in Liberia appeared to suggest that work may actually enhance trypanotolerance, although as the author himself noted his observations were based on data from a very small number of animals which may or may not have been previously exposed to trypanosomosis. This debate led Agyemang, Dwinger, Touray Jeannin, Fofana and Grieve (1990) to conclude that one of the main constraints to advocating the use of trypanotolerant livestock may actually be a lack of knowledge of the factors which can influence the stability of that tolerance.

Although trypanotolerant cattle are able survive in areas of heavy tsetse fly challenge, trypanosomosis will reduce their productivity (Murray *et al*, 1977b) including the work performance of any animals used for draught power (Bourn and Scott, 1978). Alford (1994) reported that farmers in The Gambia regard loss of power when working N'Dama as one of the clinical symptoms of trypanosomosis. The problem of working cattle with trypanosomosis has long been recognised in that country. According to Sumberg (1992) the Department of Agriculture Annual report for 1944 stated that "emphasis is laid on the use of cattle as manure makers rather than as a means to effect extensive ploughing", previous attempts to demonstrate the value of ox-drawn equipment to farmers having failed because the oxen suffered badly with trypanosomosis (Sumberg and Gilbert, 1992).

Murray (1988) suggested that nutritional status is one of the most important factors affecting the susceptibility of trypanotolerant livestock to infection with trypanosomosis. This was supported by Mortelmans and Kageruka (1976) who stated that good feeding is essential if trypanotolerant cattle are to thrive under a disease challenge. Previous studies in The Gambia have shown that the severity of infection in grazing cattle can be reduced by supplementation with small amounts of

concentrate feed (Agyemang *et al*, 1990b; Little, Dwinger, Clifford, Grieve, Kora and Bojang 1990).

The reduced susceptibility of the N'Dama to trypanosomosis compared to the susceptibility of other breeds of African cattle is considered to be due to a superior immune response (Desowitz, 1959; Morrison and Murray, 1979), coupled with physical and physiological traits such as modified skin structure and enhanced heat tolerance (Murray and Morrison, 1981), both of which reduce challenge.

The experiment described in this chapter was therefore designed to examine the immune responses of working and non-working N'Dama cattle, when challenged with a novel *Trypanosoma congolense* serodeme and to determine what effect if any plane of nutrition had on those responses, whilst also monitoring the effects of trypanosome infection on work ouput. The study was undertaken in collaboration with the International Trypanotolerance Centre (ITC), Banjul, The Gambia and the Natural Resources Institute (NRI), Chatham, UK. Elements of the experiment not reported here were part of an ITC / NRI project on management strategies to improve the productivity of trypanotolerant livestock in tsetse infested areas. The design of this experiment was by necessity, influenced by the aims of all the collaborators.

6.2 Materials & Methods

6.2.1 Location

The fieldwork for this experiment was carried out at the International Trypanotolerance Centre (ITC) headquarters, at Kerr Serigne on the Gambian coast between January and April 1994. Nutritional and haematological analyses were performed in the ITC laboratories on site. All the immunological work was carried out at the CTVM, University of Edinburgh, in Scotland.

6.2.2 The experimental design

A 2 × 2 factorial design was used to examine the effects of work and plane of nutrition on the pathogenesis of a trypanosome infection in trypanotolerant N'Dama cattle, as well as determining the immune responses of those animals to the infection and monitoring the effects of infection on work output. Two groups of cattle were worked in pairs pulling weighted sledges round a track 5 days a week, for the 12 weeks of the experiment, the other two groups were not worked. The work output of each of the working pairs was monitored daily throughout the study. One working and one non-working group were fed on a high plane of nutrition, receiving sufficient metabolisable energy (ME) to meet their estimated requirements for maintenance and work without the animals having to consume any of the low quality roughage which was offered to all animals *ad libitum*. The other two groups of cattle were fed on a basal plane of nutrition which required them to consume large quantities of the roughage just to meet their estimated energy requirements for maintenance. Four weeks after the start of the experiment all animals were artificially infected with a novel serodeme of *T. congolense*. Thereafter the cattle were worked or rested as appropriate for a further 8 weeks, while the course of the infection and the immune

responses of each animal were monitored. After 12 weeks all animals were treated with a trypanocide to eliminate the parasites.

An additional pair of cattle were worked alongside the two working groups for the duration of the experiment. These two animals acted as a control for the work output of the two infected groups and as such were not infected. They were used to monitor changes in work output over the experiment which might have been attributable to factors other than trypanosomosis; factors such as changes in climate or track surface. These control animals were fed on the high plane of nutrition throughout the study.

6.2.3 Animals

Forty N'Dama bulls were purchased from farmers on the south bank of the Gambia river in areas of low tsetse fly challenge, to provide a pool for the subsequent selection of 34 animals for the experiment. The animals purchased were 2 - 4 years old, determined from their dentition, (Miller and Robertson, 1945; Appendix 28) weighed between 130 and 180 kg and were all in similar body condition (Nicholson and Butterworth, 1985). Three extra bulls from the ITC station herd, in the same age range and in similar body condition to those purchased were added to the pool to give a total of 43 animals.

Two blood samples were collected from each bull by venepuncture of the jugular vein (Vacutainers, Becton & Dickinson Ltd., UK), one a 10 ml sample collected in a plain tube for immunological screening, the other a 5 ml sample collected in a tube containing heparin for parasitological screening. Samples were examined to determine whether any of the animals had patent trypanosome parasitaemias and / or high levels of anti- *T. congolense* antibodies, particularly

variant specific (VAT) antibodies to *T. congolense* ITC84, the stabilate to be used in the experiment (section 6.2.14).

6.2.4 Animal training

The animals were trained over a period of one month, to pull metal sledges in pairs using a head yoke attached to the horns. The bulls were controlled with reins and nose ropes, in the same way as the buffalo used in the Indonesian study (Chapter 4).

6.2.5 Animal selection and allocation to treatment groups

Thirty-four of the 43 bulls were selected for the experiment on the basis of their willingness to work and their body weight, bulls weighing less than 160 kg at the time of selection were rejected. The 34 animals chosen were divided into two weight bands light and heavy, with mean liveweights of 181 ± 1.7 kg (n = 16) and 203 ± 1.5 kg (n = 18) respectively. Four animals from each band were then were allocated at random to each of four experimental groups detailed in Table 6.1. The two remaining high weight band animals were used as the controls. Within the two working groups (BW and HW) bulls of similar liveweight were paired (Table 6.2), although some substitutions had to be made where animals refused to work together. Six of the animals selected had trypanosomal antibodies, three of those had variant specific antibodies to ITC84 as indicated in Table 6.2.

Group	No. of animals	Exercise	Nutrition	Infection
BN	8	no work	basal plane	infected
BW	8	work	basal plane	infected
HN	8	no work	high plane	infected
HW	8	work	high plane	infected
Control	2	work	high plane	not infected

Table 6.1 : Animal groups.

6.2.6 Animal husbandry

All animals were vaccinated against pasteurellosis using a vaccine prepared by the Senegalese Institute of Agricultural Research (ISRA) at a rate of 2 ml/head and were treated with ivermectin (1% w/v, Ivomec® Merck Sharp Dohme Ltd., UK) at 1 ml/50 kg body weight, against ecto and endoparasites immediately before the trial.

6.2.7 Time periods

The first day that groups BW and HW were worked was denoted as Day 1 of the experiment. For data analysis and interpretation, the study was divided into three periods:

Period 1. Pre-infection, Day 1 - 26 (weeks 1 - 4).

Period 2. Pre-patent (trypanosomes not yet detected in blood samples from all 32 bulls by the microhaematocrit centrifugation technique (MHCT) (Woo, 1970), Day 27 - 35 (week 5).

Period 3. Parasitaemic (trypanosomes observed in blood samples from all 32 bulls by MHCT), Day 36 - 84 (weeks 6 - 12).

Group	Work team	Animal no.	Liveweight at selection (kg)	Body weight band
BN	Not worked	1	160	low
		6	174	low
		7	179	low
		8	184	low
		13	191	high
		14	194	high
		15	200	high
		16	211	high
BW	Team 5	2	177	low
	Team 5	5 *	169	low
	Team 6	3	178	low
	Team 6	4	183	low
	Team 7	9	192	high
	Team 7	10	197	high
	Team 8	11	204	high
	Team 8	12	218	high
HN	Not worked	21	161	low
		22	171	low
		23	178	low
		24 *	187	low
		25	189	high
		29	189	high
		31	199	high
		32	207	high
HW	Team 1	17	163	low
	Team 1	18	176	low
	Team 2	19 **	178	low
	Team 2	20	180	low
	Team 3	26	199	high
	Team 3	30	195	high
	Team 4	27	201	high
	Team 4	28 **	207	high
CONTROL	Team 9	33 **	184	high
	Team 9	34 *	200	high

* *T. congolense* antibody positive

** *T. congolense* antibody positive with VAT antibodies to *T.congolense* ITC84

Table 6.2: Bull liveweights at selection, experimental groups and work teams.

6.2.8 Animal nutrition

A month before the study the cattle were introduced to the feedstuffs to be used. Each animal was fed 239 g DM/day of rice bran (*Oryza sativa*) and 1888 g DM/day of groundnut hay (*Arachis hypogaea*), with *ad libitum* dried andropogon grass (*Andropogon gayanus).* A fortnight before the experiment started the weights of feed offered were adjusted to the experimental rations. Groups HN, HW and control (high plane of nutrition) were fed sufficient groundnut hay and rice bran to provide them with 1.4 times their estimated metabolisable energy requirements for maintenance (ME_{maint}), groups BN and BW (basal plane of nutrition) were fed at 0.8 × estimated ME_{maint}. Rations were calculated according to MAFF (1984) recommendations using Equation 6.1 below.

$$ME_{maint} = 8.3 + 0.091W$$

Equation 6.1

where

ME_{maint} = metabolisable energy requirements for maintenance (MJ)

W = animal liveweight (kg)

Bulls in the high weight band (Table 6.2) were rationed as 200 kg animals those in the low band as 180 kg animals (180 and 200 kg were the mean weights of the two bands at that time). The actual amounts of each feedstuff offered to each group are given in Table 6.3. From day 48, 22 days post infection (p.i.) a sesame cake (*Sesamum indicum*) supplement was introduced for all animals, at 1.9 g DM/kg liveweight, fed according to the liveweight bands at the start of the trial. All animals were offered *ad libitum* dried andropogon grass throughout the experiment, chopped into 200 - 300 mm lengths. The nutrient analyses of the feedstuffs used for rationing

purposes were derived from previous experiments at ITC (Bennison, 1994) and confirmed by weekly laboratory analysis of bulked daily grab samples of the feeds (Table 6.4).

Plane of nutrition	**HIGH**	**BASAL**
Experimental groups	**HN, HW & Control**	**BN, BW**
	(g DM/kg LW)	**(g DM/kg LW)**
Rice bran	1.2	1.2
Groundnut hay	17.7	9.4
Andropogon grass	*ad libitum*	*ad libitum*
Sesame cake (from day 48)	1.9	1.9

Table 6.3 : Rations offered to the bulls in each group, per kilogram of animal liveweight (LW).

Feedstuff	**n**	**Dry matter**	**Estimated ME**	**Crude Protein**
		(%)	**(MJ/kg DM)**	**(g/kg DM)**
Rice bran	11	95.5 ± 0.28	11.0	52 ± 1.7
Groundnut hay	11	94.4 ± 0.44	10.0	81 ± 2.9
Andropogon grass	11	94.2 ± 0.62	7.4	21 ± 1.4
Sesame cake	4	96.2 ± 0.16	12.4	312 ± 16.8

Table 6.4 : Nutrient analysis of the feedstuffs used for rationing.

Bulls were fed individually. Feed was withdrawn from the non-working animals whilst the others were working to ensure that all had equal access to the feed they were offered. The cattle were watered once a day, as is the custom in The Gambia. Daily food and water intakes were recorded for each bull throughout the trial (Plate 6.1). When the bulls were not being worked they were kept tethered to individual stakes. All animals had free access to mineral blocks.

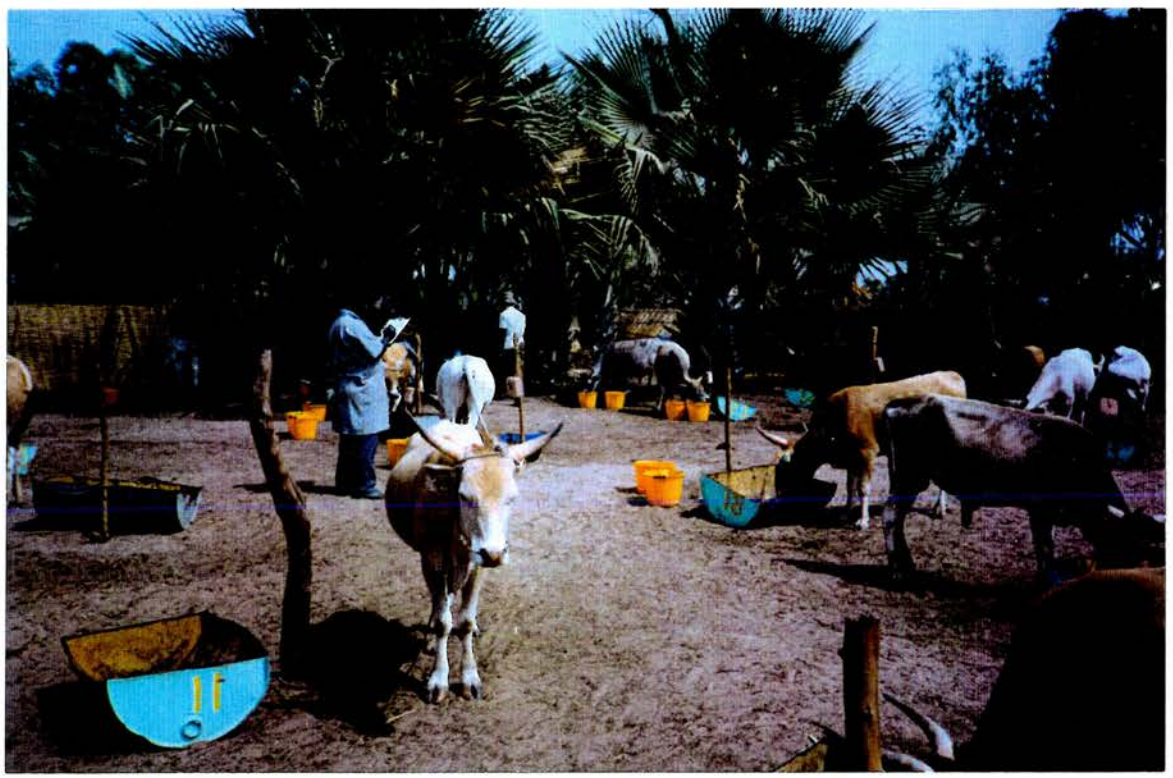


Plate 6.1: Monitoring the bulls' water intake.

6.2.9 Work

The bulls in groups HW, BW and the control were worked in pairs, matched for size and willingness to work together. Each pair pulled a similar weighted metal sledge round a 2.05 km compacted sandy loam track, completing four circuits per day, five days a week (Monday - Friday), for the twelve week duration of the trial. Details of which bulls were allocated to which teams are given in Table 6.2. Drivers were allocated to the nine teams at random, remaining with the same one for the duration of the experiment barring accident or illness. Work started between 09.00 - 09.30 hrs each morning and continued until each team had completed its four laps. Teams started at two minute intervals and were encouraged to work at their own pace, overtaking when required (Plate 6.2). If one or other animal in a team showed pre-determined signs of undue distress, that team was retired for the day.

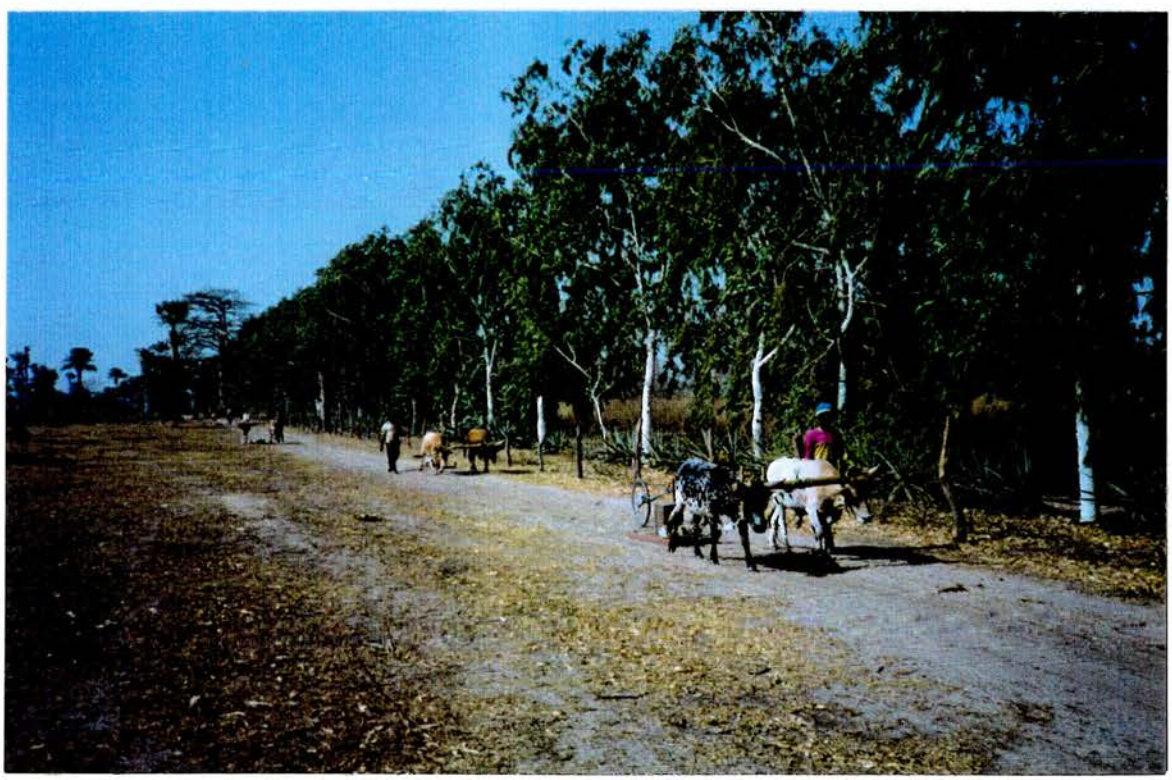


Plate 6.2 : Teams pulling their loaded sledges around the track.

Sledge loads were initially set at 17% of the combined week 0 liveweights of the two animals in the team, but were reduced to 10% in weeks two and three while the bulls became accustomed to the work. The work data from these three preliminary weeks including lap times and speeds was not used in the subsequent analysis. From week 4, sledge weights were fixed at 12.5% of week 0 team liveweight for the remainder of the experiment. The average draught force (ADF) required by each team to pull its sledge was measured immediately prior to week 4 (ADF tests), using an ergometer and load cell as described in section 3.1.1.

Criteria for retiring a fatigued team

On welfare grounds the following criteria were employed to assess whether a fatigued team should stop working:

1. If a team that took more than one hour to complete a single lap of the track (2.05 km), it was withdrawn from work for the remainder of that day. This rule was not made known to the team drivers for obvious reasons.
2. If the blood packed cell volume (PCV) of either of the bulls in a team fell to 15% or less the team was not worked for the remainder of the trial. All the bulls were blood sampled three times a week before work for the duration of the experiment.
3. If either of the bulls in a team lost more than 15% of their initial body weight, that team was not worked for the remainder of the trial.

Monitoring work output

The number of laps completed by each team and the time taken for each lap, were recorded daily throughout the trial, allowing working speeds (ms^{-1}) to be calculated. The work done (kJ) and distance travelled (m) by the control team (team 9) was measured at least once a week throughout the experiment using the ergometer

(Plate 6.3). Daily work done by teams 1 - 8 was estimated by multiplying each team's corrected ADF (3.1.1) by the distance the team walked using a standard lap length of 2056 m. The power output (Watts) of each team was calculated from the work done (Joules) divided by the time worked each day (seconds).

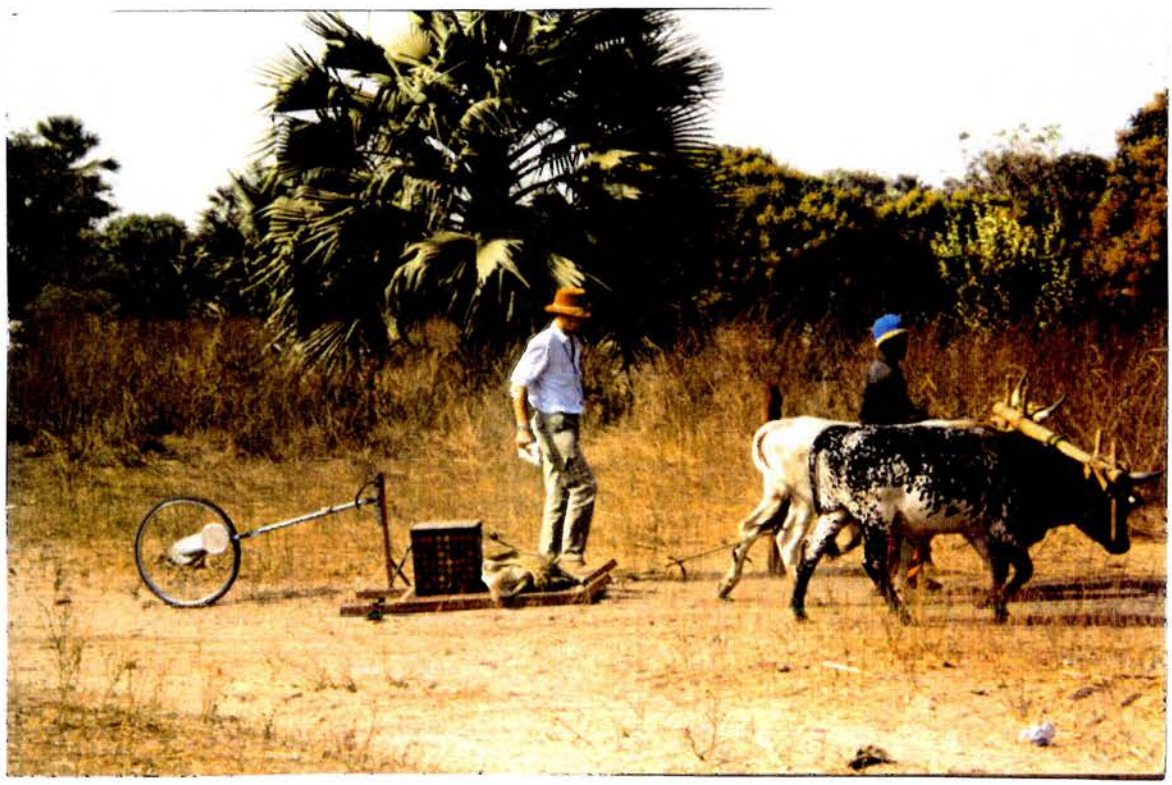


Plate 6.3 : The control team pulling their sledge with the ergometer mounted on it.

Animal energy expenditure for work

The daily net energy (NE) expenditure of each bull for work was estimated using the factorial method developed by Lawrence (1985) as detailed in section 3.1.3 using the following constants:

L = load carried (kg) = weight of the yoke, 6.9 kg

A = energy used by cattle to move 1 kg of body weight 1 m horizontally

2 J/m (Agricultural Research Council, 1980; Lawrence, 1985)

B = energy used by cattle to move 1 kg of applied load 1 m horizontally

2.6 J/m (Agricultural Research Council, 1980; Lawrence and Stibbard, 1990)

C = efficiency of cattle doing mechanical work

0.298 (Lawrence, 1985)

6.2.10 Animal liveweights

The bulls were weighed twice weekly throughout the trial using an electronic balance (Tru-Test AG500-02, Tru-Test Distributors Ltd., Auckland, New Zealand). The balance was checked for accuracy using a known weight on each occasion. A weekly mean liveweight was calculated for each animal and used in all subsequent data analysis to minimise any transient gut-fill effects.

6.2.11 Animal body temperature

The rectal temperature of each animal was recorded twice daily on working days using digital thermometers (Boots Ltd. Nottingham, UK), immediately before and after work (nominally 08.00 and 12.00 h), irrespective of whether or not it was in a working group.

6.2.12 Meteorological data

Wet and dry bulb, and maximum and minimum ambient temperatures were recorded on-site at 08.00 h and 15.00 h daily for the duration of the trial. Relative humidity was calculated from the wet and dry bulb temperatures using a psychrometric curve.

6.2.13 Infection with *T. congolense*

On day 27 all bulls with the exception of the two control animals, were infected with *T. congolense* ITC84 (Dwinger *et al*, 1992) using infected blood from three goats. The goats which had been infected one week earlier by injection of cryopreserved material, were bled from the jugular vein and the blood obtained was pooled. The blood was kept chilled (4°C) prior to injection into the cattle.

Infection was by intradermal injection, each bull receiving 0.2 ml of blood containing at least 10^4 trypanosomes /ml at each of five sites along it's left flank. The sites had been shaved and swabbed with alcohol before injection.

A stabilate was made from some of the infected goats' blood used to infect the bulls according to the method described by Murray, Trail, Turner, and Wissocq (1983). This was stored in liquid nitrogen (–70°C) and later shipped back to the UK, for use in the serological testing programme.

After infection the bulls in HW and BW were worked alongside the uninfected controls for a further 8 weeks. On day 85 all the infected bulls were treated with diminazene aceturate (Berenil, Hoechst UK Ltd., UK) at 7 mg/kg body weight, to eliminate the trypanosomes.

6.2.14 Pre-experiment screening of animals for patent infections and *T. congolense* antibodies

Parasitological screening

Heparinised blood samples collected from the 43 cattle before selection of the animals for the experiment, were screened for the presence of trypanosomes by MHCT (Woo, 1970) and by mouse inoculation (Murray *et al*, 1983). This was done by an ITC member of staff with considerable experience of the techniques.

Immunological screening

Whole blood samples collected from the 43 cattle were allowed to clot before the serum was extracted by centrifugation. The serum samples were then heat treated at 56°C for 30 minutes, to comply with Department of Agriculture & Fisheries, Scotland (DAFS) regulations governing the importation of animal pathogens, before being frozen at –20 C° and shipped to Scotland for immunological testing at CTVM.

In Scotland the imported sera were aliquoted into 0.5 ml volumes and stored at –20°C. Each sample was initially screened for the presence of *T. congolense* antibodies by indirect ELISA (Luckins, 1977) as outlined in section 3.2.1. ELISA positive samples (those with OD's more than twice the negative control) were tested for the presence of variant specific (VAT) antibodies to *T. congolense* ITC84 by agglutination test (Lumsden, Herbert, and McNeillage 1973) detailed in 6.2.19.

6.2.15 Monitoring host responses to infection

Blood sampling

All the bulls were blood sampled three times a week on Mondays, Wednesdays and Fridays from the jugular vein, commencing in week 2. Samples were always collected between 08.00 and 08.15 h to minimise the effects of any diurnal

variation in packed red blood cell volume (PCV) (Greig, Murray, Murray and McIntyre, 1979). Two, 4 - 5 ml samples were taken from each of the 32 infected bulls, one into a plain glass vacutainer, the other into a vacutainer containing EDTA. Only EDTA samples were collected from the two control bulls. Samples collected into EDTA were used for haematology and parasitology. Whole blood samples were used for serology.

Haematology

The blood samples containing EDTA were analysed on a Cobas Minos Vet Blood Analyser (Roche Products Ltd., Welwyn, UK) to determine red (RBC) and white (WBC) cell counts, haemoglobin concentrations and PCV. Weekly mean haematological values were calculated for each animal over the experiment to minimise daily fluctuations. The mean red cell volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were automatically calculated for each bull for each week of the experiment from the parameters measured.

Parasitology

Approximately 30 µl of the EDTA preserved blood from each animal was used to determine trypanosome concentration by MHCT (Woo, 1970). Trypanosomes were detected by examination of the plasma-leukocyte interface after centrifugation, quantified by the dark ground buffy coat method of Murray *et al* (1977a) and scored according to Paris, Murray and McOdimba (1982). Counts were done by the same person throughout the trial to ensure consistency, one of the ITC staff with considerable experience of the technique.

The following parasitaemia parameters were calculated for each bull; number of samples collected before trypanosomes were first detected (prepatent samples); peak parasitaemia score attained; mean parasitaemia score after first appearance of trypanosomes; number of samples when the score was 5+ or higher; and the rate of decline of parasitaemia after the peak score. The latter was estimated by linear regression using the least squares technique to fit a straight line to the parasite scores for each animal. The gradient of the line was taken as a measure of the rate of decline of parasitaemia.

Serology

Serum extracted from the whole blood samples which had been allowed to clot, was aliquotted into 800 µl volumes and stored at −70°C, for subsequent determination of circulating antibody levels (IgG_1, IgG_2, IgM and total IgG) by ELISA in the UK. Prior to export the serum was heat treated to 56°C for 30 minutes to comply with Department of Agriculture & Fisheries, Scotland (DAFS) regulations.

6.2.16 Determination of circulating antibody levels by ELISA

Indirect antibody ELISA's as described in section 3.2.1, were used to evaluate immune responses to trypanosomal challenge in the 32 animals infected during the experiment. Individual assays were developed to quantify parasite specific IgG_1, IgG_2, IgM and total IgG. An extra step was used in the IgG_1 and IgG_2 assays (indirect double antibody sandwich ELISA's) to increase the sensitivity and because labelled class specific anti-bovine immunoglobulin preparations were not readily available. The basic methodology used for each ELISA is detailed in sections 3.2.1 and 3.2.2.

Serum samples collected from individual animals over the course of the experiment were examined to establish immunoglobulin profiles as infections progressed. Immunoglobulin titres were compared between groups on specific dates to determine the effects of work and plane of nutrition on immune response.

6.2.17 ELISA techniques

Preparation of antigenic material for ELISA's

Trypanosomal antigens for the pre-experiment screening ELISA and for the subsequent ELISA's to determine circulating antibody levels p.i. were prepared in the same way although different trypanosome stabilates were used. The antigen for the screening assay was prepared from *T. congolense* stabilate 2193, while that for the assays to monitor parasite-specific antibodies post-infection was prepared from the stabilate used to infect the bulls, *T. congolense* ITC84. In both cases trypanosome populations were expanded from cryopreserved stabilates by sequential passage in groups of laboratory rodents. Once parasitaemias exceeded 50 trypanosomes per microscope field in wet blood films examined at × 400 magnification the rodents were ex-sanguinated by cardiac puncture under halothane anaesthesia. The infected blood was collected in heparin (10 i.u./ml of blood) and the trypanosomes separated from the blood elements by anion exchange chromatography using diethylaminoethyl cellulose (DE52, Whatman International Ltd. Kent, England) according to Lanham and Godfrey (1970). After centrifugation at 3000 *g* for 30 minutes at 4°C (Mistral 300 MSE, Chill-spin) the resultant trypanosome pellet was washed three times by resuspension in phosphate buffered saline (PBS) pH 7.4, before a final centrifugation at 8500 *g* (Biofuge A, Heraeus, Sepatech Ltd). The pellet was then resuspended in the minimum volume of PBS consistent with the efficient removal of the trypanosome suspension, before storage at –20°C.

Additional passages and separations were carried out producing further pellets until sufficient trypanosomes had been separated for the ELISA programmes. The frozen pellets were then pooled and a cell lysate prepared by three rapid freeze / thaw cycles, using dry ice (–70°C) and warm running water (40°C), according to Authié, Muteti and Williams (1993b). The resultant lysate was centrifuged (Biofuge A, Heraeus, Sepatech Ltd) at 12000 *g* for 2 minutes and the supernatant drawn off, aliquoted in 50 µl volumes and stored at –20°C until required.

Establishing the parameters for individual ELISA's

To establish working dilutions for the various components of each ELISA, a series of chequerboard titrations were carried out for each assay as detailed in section 3.2.3.

Positive and negative control sera

Positive and negative control sera were included on all plates to monitor plate to plate variations. Serum collected from a Friesian cow during a previous CTVM experiment and known to contain high levels of *T. congolense* antibodies, was used as the positive control for the pre-experiment animal screening ELISA. Serum from an N'Dama bull at ITC, similar to the trial animals, kept on the high plane of nutrition and infected with *T. congolense* ITC84, was used as the positive control for the ELISA's measuring class specific immunoglobulin responses to trypanosome infection. The serum was collected on two occasions more than 28 days p.i. and pooled. Serum samples were also collected from ten apparently healthy European cattle kept at CTVM in Scotland. The serum giving the lowest OD reading at a wavelength of 450 nm on indirect antibody ELISA for *T. congolense* antibodies, was selected as the negative control for all assays.

Procedures used to minimise variations within an ELISA

Nine basic procedures described in section 3.2.4, were adopted to minimise plate to plate and day to day variations in each assay, allowing results to be compared across plates. Wherever possible however, serum samples for comparison were tested on the same plate. Consequently two plate layouts were used, one in which all the sera taken from an animal over the course of the experiment were screened on a single plate the other in which all the sera collected on a single day were screened on one plate.

Techniques used for correcting inter-plate variation

The effectiveness of two techniques for correcting results for plate to plate variation were assessed; *viz* correction based on a calibration factor (F) derived from positive and negative control sera (Luckins, 1983) as used in the studies on sheep immune responses (Chapter 5); and correction using calibration lines plotted from the OD results of serial dilutions of positive control serum (de Savigny and Voller, 1980; Kemeny, 1992) described below.

To assess the effectiveness of the two techniques sera collected from six of the experimental bulls (chosen at random) over the course of the experiment were tested across a number of plates and the results for each bull obtained using each correction technique were compared with the results when the same samples were screened on a single plate. The comparison was undertaken for both the IgG_1 and IgM assays. The F values calculated for each plate in each assay used in the study are given in Appendices 5, 6 and 7.

Calibration lines

To produce calibration lines, eight serial dilutions of the positive control serum, spanning the dilution used for the test sera, were tested on each plate. These dilutions were made up as a single batch, aliquoted into the quantities needed for a

single day's testing and then stored at -20°C until required. Each dilution was tested in triplicate and the mean OD calculated, on the basis that the anti-$\log_{10}$ of the highest and lowest values did not differ from the anti-$\log_{10}$ of the median value by more than 10% (Scott, 1994). If either of the values was outside this range the aberrant value was deleted as inaccurate. Each dilution was expressed as an arbitrary activity value (AV) for example a dilution of 1:50 might be given an AV of 125, consequently a dilution of 1:25 would then have an AV of 250, 1:100 an AV of 62.5. Mean OD was then plotted against $\log_{10}$ of activity and a line of best fit was calculated by the least squares method of linear regression analysis. Regression lines with correlation coefficients of less than 0.95 were plotted and checked; if the calibration line was clearly non-linear correction by this method was abandoned; if the low correlation coefficient was due to a single aberrant value, that value was deleted. OD's from the test sera were expressed as AV's by reading off the appropriate $\log_{10}$(AV) from the calibration line, calculating the anti-$\log_{10}$ of that value and multiplying it by the sample dilution factor. The dilution factor equalled the dilution of the test sera divided by the dilution of the most concentrated positive control used to derive the calibration line. An example of the methodology is given in Appendix 29.

6.2.18 Details of specific ELISA's

Pre-experiment ELISA used to screen cattle for trypanosome infection prior to the study

An indirect ELISA was used to screen serum from each of the 43 animals prior to the experiment to determine if any of the bulls already possessed *T. congolense* antibodies. Animals were considered positive if the mean OD of the duplicate samples tested was more than twice that of the negative control on the plate. Samples producing poor duplicate OD values (those differing by more than 10% of the anti-$\log_{10}$ of the larger) on the first or second plate were tested a third time. The

components of the assay and the dilutions at which they were used are given in Table 6.5.

Assays to establish immunoglobulin profiles following infection

Indirect double antibody sandwich ELISA's were used to measure trypanosome specific IgG_1 and IgG_2 levels after infection, each with a class specific monoclonal antibody. Parasite specific IgM and total IgG levels in the bovine sera were assayed by indirect antibody ELISA's using class specific conjugated antibody preparations which obviated the need for the extra layer. Class specific conjugate preparations were not readily available for the IgG_1 and IgG_2 assays. Details of the components of all four assays together with the dilutions employed are presented in Table 6.5.

6.2.19 Agglutination testing

Mouse blood infected with *T. congolense* ITC84 was centrifuged at 3000 g for 3 minutes at 4°C to remove the red blood cells. The plasma was then drawn off and centrifuged for a second time at 8500 *g* for 3 minutes to concentrate the trypanosomes and reduce the plasma volume, giving a minimum concentration of 1×10^8 trypanosomes /µl of plasma.

For antibody screening doubling dilution series of the bovine sera to be tested were prepared in PBS, from an initial dilution of 1:2. Each dilution was pipetted into duplicate wells of a 60 well Terasaki pattern microtitre plate (Nunc Ltd. distributed by Life Technologies Ltd, Paisley) that had previously been flooded with a small volume of mineral oil. 2 µl of the trypanosome infected mouse plasma was then added to each well using a Hamilton auto-dispenser and the plate was incubated at room temperature for 30 minutes. After incubation each well was examined for

ELISA	Pre-expt. screening	Total IgG	IgM	IgG_1	IgG_2
Type of assay	IA	IA	IA	IDAS	IDAS
Trypanosomal antigen	2193	ITC84	ITC84	ITC84	ITC84
dilution	1:1000	1:1600	1:1600	1:1600	1:1600
Serum dilution	1:200	1:100	1:30	1:25	no dilution established
Monoclonal antibody	—	—	—	Mouse anti-bovine IgG_1 [b]	Mouse anti-bovine IgG_2 [b]
dilution	—	—	—	1:50	no dilution established
Conjugate	Rabbit anti-bovine IgG (whole molecule) horseradish peroxidase [a]		Sheep anti-bovine (μ chain) IgM horseradish peroxidase [c]	Goat anti-rabbit IgG (whole molecule) horseradish peroxidase [a]	
dilution	1:12000	1:12000	1:12000	1:9000	no dilution established

IA	Indirect antibody ELISA
IDAS	Indirect double antibody sandwich ELISA
Trypanosomal antigen	*T. congolense* isolate used to make the crude lysate antigen

[a] Sigma Chemical Co., St. Louis, USA
[b] Serotec Ltd., Oxford, UK
[c] The Binding Site Ltd., Birmingham, UK

Table 6.5 : The dilutions used for the various ELISA's performed to monitor specific immunoglobulins in the bulls on the experiment.

agglutinated trypanosome cells, indicating the presence of variant (VAT) specific antibodies to *T. congolense* ITC84.

6.2.20 Data analysis

Nutrition data

Mean daily dry matter intakes (DMI) of each feedstuff were calculated for each bull, for each week of the experiment. For comparative purposes groundnut hay intakes were expressed as percentages of feed offered, andropogon which was fed *ad libitum* as grams of dry matter consumed per kilogram of metabolic liveweight per day (g DM/$kg^{0.75}$/day). Groundnut hay data being percentage data was transformed before analysis (section 3.3). Andropogon intake data from one of the bulls in HN was excluded from the data set analysed as the results were very different from those for all the other animals. Intakes of rice bran and sesame cake were not statistically analysed as they did not reflect voluntary intakes.

A series of ANOVA's were used to compare group differences in andropogon intakes each week throughout the experiment. Within the groups, paired T-tests were used to determine what effect infection had on intakes of both andropogon grass and groundnut hay by comparing the mean pre-infection intake (weeks 1 - 4) of each bull with its intake week by week p.i..

Mean daily ME intakes per unit of metabolic liveweight were calculated for each bull for each week of the experiment. The effects of infection on ME intake within each group were tested in exactly the same way as the groundnut and andropogon intakes, using a series of paired T-tests.

Lap times of working animals

Only data from weeks 4 to 7 inclusively was subjected to statistical analysis. Before week 4 sledge loads were not fixed and after week 7 some of the teams were withdrawn from work so that there were insufficient replicates in each group for reliable analysis. The effect of infection on lap times was examined by ANOVA comparing weekly mean lap times for each team in weeks 4 to 7. Balanced multi-way ANOVA's were used to identify significant group (HW and BW), day of the week and lap effects on lap time together with any interactions, within each of the three periods of the experiment, pre-infection, prepatent and parasitaemic. Lap times for the control group were not included in these analyses as they were only from a single team and more importantly, included a large number of missing values.

Speeds of working animals

The weekly mean speeds of each team in weeks 4 and 8 were compared using one-way ANOVA's.

Power outputs

The mean power output of each team each week p.i. was expressed as a percentage of the week 4 output of the team. This data was not subject to statistical analysis.

Animal liveweights

Differences in animal liveweight between groups at the start of the experiment (week 0), immediately prior to infection (week 4) and at the end of the experiment (week 12), were tested for significance using a one-way ANOVA. A two-way ANOVA was used to identify dietary and work effects on each of these occasions.

Liveweight changes over periods 1 and 3 (pre-infection and parasitaemic) were analysed in a similar manner, looking firstly for any group effects and thereafter for nutritional and work effects.

Body temperatures

The daily body temperatures of each bull pre- and post infection were compared using Mann Whitney one tailed tests, with separate analyses for 08.00 and 12.00 hrs temperatures and for temperature change over the working day. Group median temperatures at 08.00 and 12.00 hrs, together with the median temperature change over the working day were calculated for each period of the experiment from the median values for each bull. Kruskal Wallis tests were used to compare these group medians. Median values for the control group were excluded from the analysis as they were derived from only two replicates.

Meteorological data

Diurnal fluctuations in ambient temperature, dry bulb temperature and relative humidity at 08.00 and 12.00 hrs daily were analysed using one way ANOVA's to determine whether the climate differed significantly between periods.

Haematological parameters

Blood parameters were tested to determine whether there were significant differences between groups, at the start and end of the pre-infection period and at the start, middle and end of the parasitaemic period (weeks 2 and 4, and weeks 6, 9 and 12, respectively). Data for weeks other than these was analysed only where graphs indicated marked differences between groups. Means of thrice weekly haematological values were used for statistical analysis to minimise the effects of any transient

fluctuations caused by such factors as poor blood sampling technique, incorrect handling of samples or insufficient watering of the animals which may have occurred. ANOVA's were used to test the data for significant differences with the exception of the WBC data in weeks 2, 4 and 6 which were not normally distributed and hence were analysed using Kruskal Wallis tests. Parametric tests were used to analyse the PCV data, because although the results were percentages they were normally distributed and the variances of the group means were not significantly different, furthermore none of the values approached 0 or 100%. Although it is not strictly correct, parametric tests are often used to used to analyse PCV data for example by Little *et al*, (1990), Paling, Moloo, Scott, McOdimba, Logan-Henfrey, Murray, and Williams (1991) and Dwinger *et al* (1992) as values seldom approach zero or one hundred percent.

Two-way ANOVA's were used to examine the effects of diet and work on PCV, RBC and haemoglobin levels. The least squares method of linear regression was used to determine if there was a significant linear relationship between a bull's PCV at the start and end of the experiment. It was also used to calculate a rate of decline of PCV for each bull between weeks 5 and 9. These rates of decline of PCV were then examined by ANOVA for significant differences between groups, and for work and nutritional effects.

The MCH and MCHC data were not statistically analysed as they were derived from RBC's, PCV's and haemoglobin levels.

Parasitological parameters

Parasitological parameters were tested for significant differences between groups using ANOVA's with the exception of the mean parasitaemia scores of the bulls once infected, which were analysed using a Kruskal Wallis test. The effects of

work and nutrition on these parameters excluding the mean parasitaemia scores, were tested using a two-way ANOVA.

ELISA data

All ELISA OD results were analysed using non-parametric tests as OD data is not normally distributed (Tijssen, 1985).

Techniques for correcting inter-plate variation

The significance of differences between OD results from multiple plates corrected using the F factor and results obtained from screening the same sera on a single plate were tested for both IgG_1 and IgM assays using Wilcoxon's signed rank test (Sokal and Rohlf, 1981). Results obtained using the calibration curve method of correction for plate to plate variation were compared with results from single plate assays and F factor corrected multiple plate assays by plotting the three sets of data on a single graph; activity value or OD against the day of the experiment when the serum was collected.

ELISA's to measure parasite-specific immunoglobulin levels

For each ELISA the median OD of each bull's pre-infection serum samples was taken as an estimate of its pre-infection anti-*T. congolense* (ITC84) immunoglobulin level. A series of Kruskal Wallis tests were used to compare the immunoglobulin levels of each group pre-infection and on each sampling day p.i. Using this approach only results produced on a single ELISA plate were compared statistically, removing the need to correct for inter-plate variation. F factor corrected results from multiple plates were used merely to determine the pattern of responses relative to point of infection.

6.3 Results

6.3.1 Pre-experiment screening of bulls

Parasitological results

Two of the bulls purchased were *T. vivax* positive on dark ground examination of wet blood smears (Murray *et al*, 1977a). These animals were treated with diminazene aceturate (Berenil, Hoechst AG., Frankfurt, Germany) at 7 mg/kg liveweight to eliminate the infections. None of the bulls proved trypanosome positive on mouse inoculation.

Immunological results

Fourteen of the 43 bulls screened had *T. congolense* antibodies by ELISA. Five of these were found to have variant specific antibodies to *T. congolense* ITC84 by agglutination testing. Although the original intention was to exclude *T. congolense* antibody positive animals from the experiment, because of the limited number of bulls available six antibody positive bulls had to be included as follows:

(a) HN and BW, one ELISA positive bull in each group.

(b) HW, two ELISA positive, agglutination test positive bulls.

(c) Control group, both bulls ELISA positive, only one agglutination test positive.

These animals are identified in Table 6.2.

6.3.2 Other disease problems

In week 3, two of the working animals were diagnosed as having bovine ephemeral fever. They suffered from high transient fevers, diarrhoea, loss of appetite and were generally listless and stiff. Bull 20 (HW) fell sick on day 16, bull 34 (control) on day 18. Neither animal was worked for the remainder of that week. Both were temporarily replaced by spare animals not part of the experiment, so that their team-mates could continue working. The sick animals were treated with long acting Tetracycline, 1 ml/10 kg LW (Hoechst A.G.). Bull 34 started working again on day 22, bull 20 a day later (day 23).

6.3.3 Animal live weight changes

Trypanosomosis caused significant weight loss. The mean weight losses of the animals in the infected groups were between 4.7 and 11.5 kg (or 110 - 270 g/d) once the bulls became parasitaemic (period 3), whereas the uninfected controls gained an average of 13.0 kg (310 g/d) over the same six week period (Table 6.6). Weight losses caused by trypanosomosis were compounded by work. Animals in the infected working groups (BW and HW) lost almost twice as much weight as their non-working contemporaries in period 3, although the difference was not significant (Table 6.7). Diet had no effect on weight change once the bulls were parasitaemic.

There were significant differences in the group mean liveweight changes over period 1, however most of this was accounted for by weight losses during the first week of the experiment, as shown in Table 6.6 and illustrated in Figure 6.1. Group liveweight changes over weeks 1 - 4 did not differ significantly. Similarly neither diet nor work had a significant effect on liveweight change over weeks 1 - 4 (Table 6.7).

Group mean liveweights at the start and finish of the experiment and immediately prior to infection are given in Table 6.8. The control bulls gained a mean

of 9% of initial liveweight over the experiment whereas the infected groups lost between 1 and 10% (Figure 6.1).

Group	Mean liveweight changes (g/day) with s.e.					
	Period 1 (week 4 LW – initial LW)	**(n)**	**Period 1 (week 4 LW – week 1 LW)**	**(n)**	**Period 3 (week 12 LW – week 6 LW)**	**(n)**
BN	−60 ± 38	7	50 ± 84	7	−110 ± 40	8
BW	−200 ± 38	8	40 ± 57	8	−270 ± 76	8
HN	140 ± 34	8	180 ± 58	8	−160 ± 51	8
HW	−90 ± 44	8	180 ± 85	8	−220 ± 54	8
Control	70 ± 36	2	220 ± 167	2	310 ± 96	2
F	11.68		1.01		5.80	
P_{group}	<0.001 ***		0.42 ns		0.001 ***	
s.e.d.(n_1,n_2)	108 (8, 8)				163 (8,8)	
s.e.d.(n_1,n_2)	112 (8, 7)				258 (8,2)	
s.e.d.(n_1,n_2)	171 (8, 2)					
s.e.d.(n_1,n_2)	174 (7, 2)					

Table 6.6 : Mean liveweight (LW) changes for each group pre-infection (period 1) & once parasitaemic (period 3), with ANOVA's to determine significant differences between groups.

There were considerable differences in the weight changes of individual animals over the experiment. Two bulls in BW lost at least 16% of their initial liveweight, while another bull in the same group lost only 3%. In addition to the two uninfected bulls, five infected animals actually gained weight; one each in BN and HW, three in HN. The maximum gain amongst the five infected animals was 7% of initial liveweight over the 12 weeks. Weights of individual animals are given in appendix 30.

Treatment	Mean liveweight change (g/day) with s.e. Period 1 (week 4 – initial LW)	(n)	Period 1 (week 4 – week 1 LW)	(n)	Period 3 (week 12 – week 6 LW)	(n)
Low nutrition	−140 ± 32	15	50 ± 48	15	−190 ± 46	16
High nutrition	30 ± 40	16	180 ± 50	16	−190 ± 37	16
Non-working	50 ± 37	15	120 ± 51	15	−130 ± 32	16
Working	−150 ± 31	16	110 ± 53	16	−250 ± 45	16
Nutrition	F = 16.74		F = 3.62		F = 0.01	
$P_{nutrition}$	<0.001 ***		0.07 ns		0.93 ns	
Work	F = 23.23		F = 0.01		F = 3.92	
P_{work}	<0.001 ***		0.93 ns		0.06 ns	
Interaction	F = 1.44		F = 0.01		F = 0.77	
$P_{nutrition \times work}$	0.24 ns		0.93 ns		0.39 ns	

Table 6.7: The effects of diet and work on mean liveweight (LW) changes over periods 1 & 3, with results of two-way ANOVA's to determine significant differences.

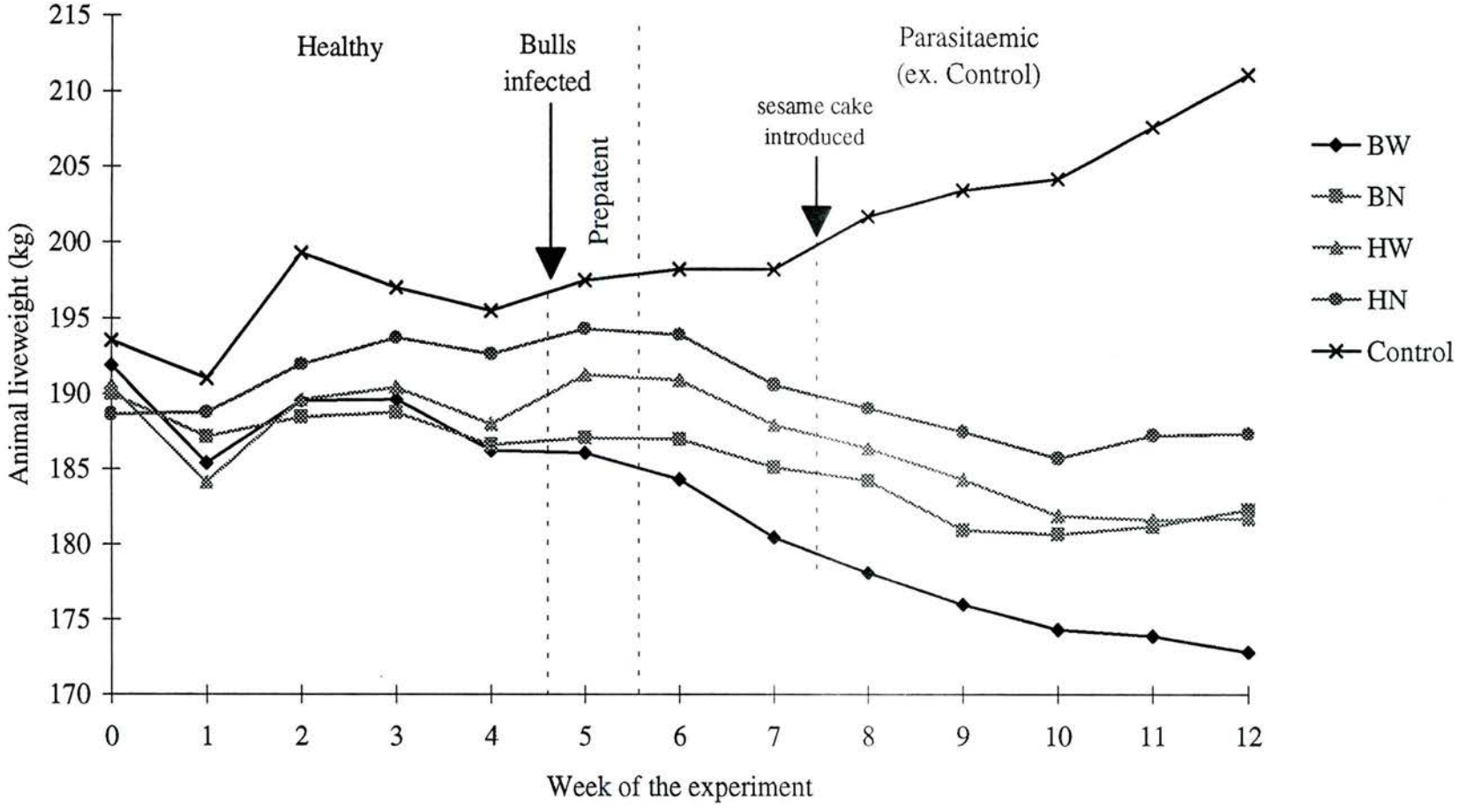
The mean initial liveweights of the bulls on the two planes of nutrition were not significantly different nor was there a significant difference at 4 or 12 weeks (Table 6.9), although by week 12 the mean liveweight of the bulls on the high plane of nutrition was 7 kg more than that of the bulls on the low plane. Similarly there were no significant differences between the mean liveweights of the working and non-working animals on any of these three occasions, however by the end of the experiment, non-working animals weighed an average of 8 kg more than their working contemporaries.

Group	(n)	Initial liveweight Week 0 (kg)	(%)	Pre-infection liveweight Week 4 (kg)	(%)	Final liveweight Week 12 (kg)	(%)
BN	8[a]	190 ± 6.1	100	187 ± 5.9	98	182± 6.6	96
BW	8	192 ± 4.1	100	186 ± 4.6	97	173 ± 5.5	90
HN	8	189 ± 4.3	100	193 ± 4.6	102	187 ± 4.5	99
HW	8	191 ± 4.8	100	188 ± 4.9	99	182 ± 3.5	95
Control	2	194 ± 9.5	100	196 ± 8.5	101	211 ± 12.3	109
F		0.09		0.38		3.03	
P_{group}		0.99 ns		0.82 ns		0.03 *	
s.e.d.(n_1,n_2)						7.31 (8,8)	
s.e.d.(n_1,n_2)						11.57 (8,2)	

[a] n = 7 for group BN initial LW only, as one the weight of one animal was missing

Table 6.8 : Group mean liveweights in kilograms and as percentages of initial weight at the start of the experiment, immediately before infection and at the end with an ANOVA of group differences on each occasion.

Figure 6.1 : Mean liveweight of each group for each week of the experiment.



Treatment	Initial LW Week 0 (kg)	(n)	Pre-infection LW Week 4 (kg)	(n)	Final LW Week 12 (kg)	(n)
Low nutrition	191 ± 3.4	15	186 ± 3.6	16	178 ± 4.3	16
High nutrition	190 ± 3.1	16	190 ± 3.3	16	185 ± 2.8	16
Non-working	189 ± 3.5	15	190 ± 3.7	16	185 ± 3.9	16
Working	191 ± 3.0	16	187 ± 3.2	16	177 ± 3.3	16
Nutrition	F = 0.08		F = 0.59		F = 1.86	
$P_{nutrition}$	0.78 ns		0.45 ns		0.18 ns	
Work	F = 0.15		F = 0.25		F = 2.17	
P_{work}	0.70 ns		0.62 ns		0.15 ns	
Interaction	F = 0.00		F = 0.18		F = 0.14	
$P_{nutrition \times work}$	1.00 ns		0.68 ns		0.71 ns	

Table 6.9 : Mean effects of diet and work on animal liveweight at three points during the experiment, with results of two-way ANOVA's.

6.3.4 Work

Average draught force results

The ADF required by team 9 to pull its sledge remained constant at 258 ± 1.8 N (n = 38) over weeks 4 - 12 as the track surface did not alter noticeably. This was a 4.7% increase on the team 9 ADF test result recorded immediately prior to week 4. The ADF test results of the other 8 teams were consequently increased by 4.7% when estimating team work outputs. Results of the week 4 ADF tests are given in Table 6.10.

Team	Team liveweight in week 0 (kg)	Sledge weight (kg)	ADF req'd to pull the sledge (mean ± s.e.) (N)	(n)	ADF as a percentage of team liveweight (%)
1	348	43.5	223 ± 0.8	15	6.4
2	366	45.8	239 ± 0.7	20	6.5
3	407	50.9	248 ± 0.8	20	6.1
4	403	50.4	261 ± 0.7	22	6.5
5	357	44.6	238 ± 0.7	16	6.7
6	373	46.6	245 ± 0.7	21	6.6
7	397	49.6	262 ± 0.8	20	6.6
8	408	51.0	260 ± 0.8	22	6.4
9	387	48.4	247 ± 0.7	20	6.4
Mean ± s.e.				9	6.5 ± 0.14

Table 6.10 : Team and sledge weights with the consequent draught forces required to pull the sledges, measured prior to week 4.

Track length

The mean length of the track measured using the ergometer attached to the team 9 sledge was 2056 ± 0.5 m/lap (n = 43).

Work patterns

Only three of the eight infected teams were able to complete the full twelve weeks work, two on the basal level of nutrition and one on the high level. Before infection (weeks 1 to 4) all teams worked well, easily completing four laps of the track each day, a total distance of 8224 m. The uninfected control team continued to do so for the remainder of the experiment, getting progressively fitter as indicated by increasing weekly mean speeds (Figure 6.2). In marked contrast however, five of the infected teams were retired early, when the PCV of one or other animal in the team dropped to 15% or less (Criteria for retiring a fatigued team, Section 6.2.9). The first team to be retired, stopped work less than three weeks after infection (Table 6.11).

The control team although easily capable of working every day during the study only actually worked for 51 of the 57 days because the driver was required for one of the infected teams on the other 6 days, when other drivers were absent.

Team	Group	Total no. of days worked	Last week worked
1	HW	43	9
2	HW	36	8
3	HW	52	11
4	HW	57	Completed all 12 weeks
5	BW	35	8
6	BW	34	7
7	BW	57	Completed all 12 weeks
8	BW	57	Completed all 12 weeks
9	Control	51	Completed all 12 weeks

Table 6.11 : Days worked by each team during the experiment out of a possible total of 57 days.

Lap times & speeds

Infection resulted in a fall in weekly mean working speeds of up to 48% as lap times increased. The eight infected teams slowed down markedly once the bulls became parasitaemic although the differences were not significant until week 7 (Table 6.12). In contrast the weekly mean lap times of the control team remained almost constant at between 26 and 27 minutes from weeks 4 to 7. By week 8, four weeks after infection, the speeds of the infected teams that were still able to work, had fallen by an average of 30% compared to their speeds in week 4 immediately before infection. Nutrition had no significant effect on lap times in any of the three periods (Table 6.13).

Week	Period	mean (mm:ss)	s.e. (mm:ss)	(n)
4	1 (Uninfected)	27:19	00:44	8
5	2 (Pre-patent)	26:20	00:32	8
6	3 (Parasitaemic)	29:52	01:16	8
7	3 (Parasitaemic)	36:46	01:57	8
F		14.23		
P_{week}		< 0.001 ***		
s.e.d.		01:46		

Table 6.12 : Weekly mean lap times for the eight infected teams for the four weeks when all teams were working with ANOVA results.

During each working day teams slowed down on each successive lap after the first, irrespective of group, period or day of the week (Table 6.14) and once parasitaemic the bulls slowed down significantly over the course of each week, Monday to Friday (Table 6.15). Only once during the experiment did an infected team have to be retired without completing 4 laps having taken more than an hour to

complete an earlier lap, although on a number of occasions teams took in excess of one hour to complete their final lap.

Plane of nutrition	Period 1	Period 2	Period 3
Basal (BW)	26:47 ± 00:12	26:42 ± 00:20	33:38 ± 00:43
High (HW)	27:51 ± 00:37	25:58 ± 00:26	33:00 ± 00:45
F	0.51	0.44	0.04
$P_{nutrition}$	0.50 ns	0.53 ns	0.85 ns
s.e.d.	01:30	01:07	03:15
(n)	80	80	160
Control	26:53 ± 00:21	26:00 ± 00:17	26:42 ± 00:21
(n)	20	13	32

Table 6.13 : Mean lap times (mm:ss) for each group in each period, with results of ANOVA's to determine the effects of plane of nutrition on lap times in each period.

Lap	Period 1	Period 2	Period 3
1	26:00 ± 00:18	25:34 ± 00:23	31:28 ± 00:47
2	27:13 ± 00:36	26:18 ± 00:40	33:05 ± 00:59
3	27:56 ± 01:02	26:08 ± 00:26	34:36 ± 01:13
4	28:08 ± 00:21	27:21 ± 00:38	34:07 ± 01:05
F	3.04	5.41	4.23
P_{lap}	0.03 *	0.002 **	0.006 **
s.e.d.	00:47	00:27	00:57
(n)	40	40	80

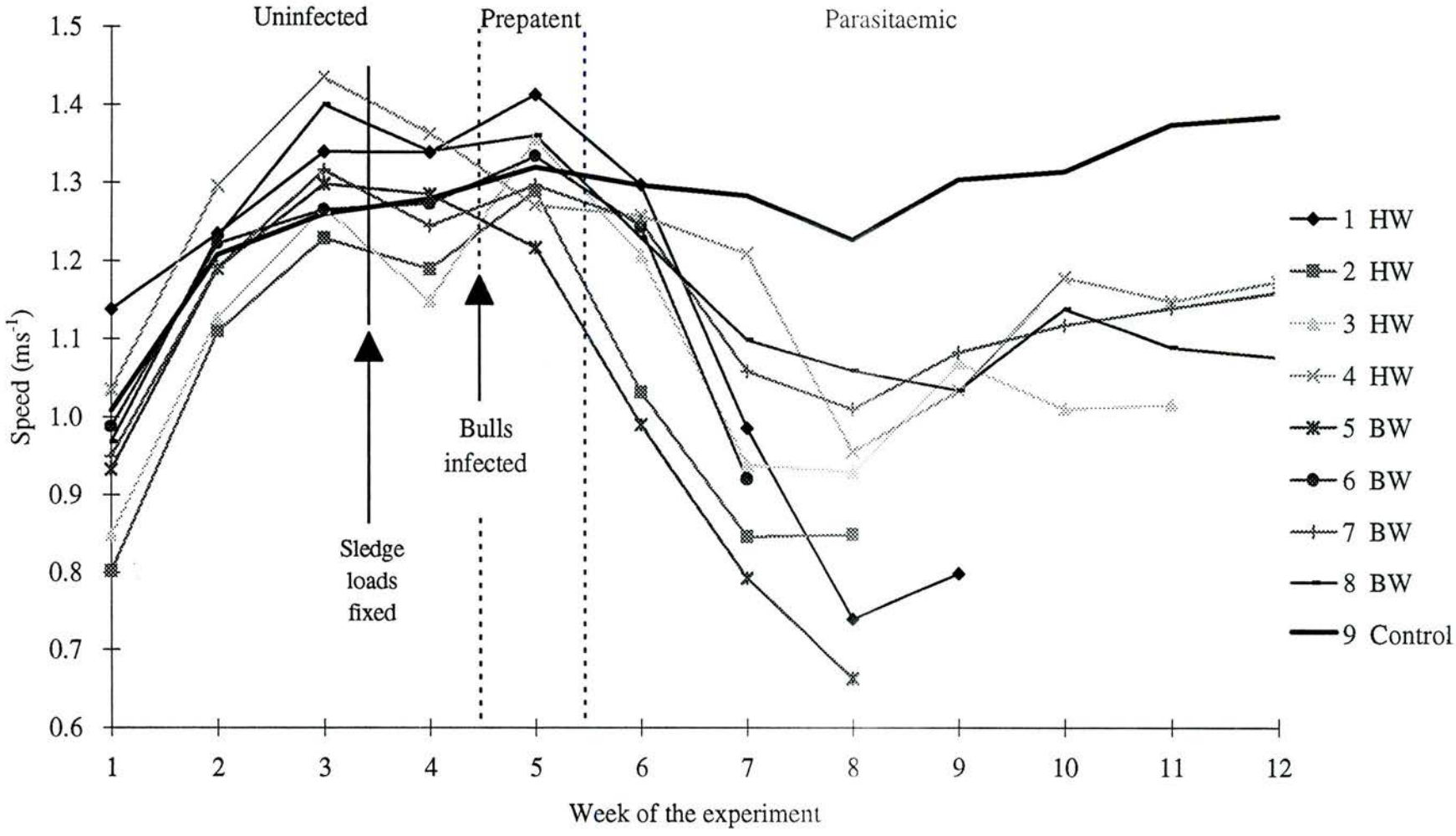
Table 6.14 : Mean lap times (mm:ss) for the eight infected teams for each lap within each period with ANOVA results.

Figure 6.2 shows the marked decline in the speeds of teams 1 - 8 once they became parasitaemic, particularly of those teams which were subsequently withdrawn from work. Working speeds increased over the first three weeks of the experiment as sledge loads were adjusted. From week 4 onwards loads were constant. In week 4 although there were significant differences between the speeds of some teams, no single team was significantly faster or slower than all others (Table 6.16). By week 8 however, the uninfected control team was significantly faster than all the infected teams, whilst teams 1 and 5 were significantly slower than all others. Teams 3, 4, 7 and 8 which continued working for most or all of period 3, speeded up again after week 8. The control team worked progressively faster throughout the experiment. Weekly mean lap times and speeds for each team over the experiment are given in appendices 31 and 32.

Day	Period 1	Period 2	Period 3
Mon	26:53 ± 00:18	27:24 ± 00:37	29:10 ± 00:27
Tues	26:49 ± 00:26	25:54 ± 00:14	30:15 ± 00:40
Wed	26:55 ± 00:54	25:55 ± 00:31	32:40 ± 00:49
Thurs	28:33 ± 01:10	25:33 ± 00:22	36:27 ± 01:36
Fri	27:27 ± 00:25	26:54 ± 01:00	38:02 ± 01:23
F	1.03	0.62	13.17
P_{day}	0.41 ns	0.65 ns	<0.001 ***
s.e.d.	01:02	01:24	01:30
(n)	32	32	64

Table 6.15 : Mean lap times (mm:ss) for the infected teams for each day of the week within each period with ANOVA results.

Figure 6.2 : Weekly mean working speed of each team over the experiment.



Team	Week 4 ($m\ s^{-1}$) (mean ± s.e.)	(n)	Week 8 ($m\ s^{-1}$) (mean ± s.e.)	(n)	Week 8 speed as a % of week 4
1 HW	1.34 ± 0.024	20	0.74 ± 0.039	14	55
2 HW	1.19 ± 0.013	20	0.85 ± 0.037	8	71
3 HW	1.15 ± 0.050	20	0.93 ± 0.014	16	81
4 HW	1.36 ± 0.024	20	0.96 ± 0.033	16	70
5 BW	1.28 ± 0.018	20	0.66 ± 0.040	3	52
6 BW	1.27 ± 0.022	20	Ret'd (Wk 7)		
7 BW	1.24 ± 0.013	20	1.01 ± 0.023	10	81
8 BW	1.34 ± 0.020	20	1.06 ± 0.023	7	79
9 Control	1.28 ± 0.017	20	1.23 ± 0.016	16	96
P_{team}	<0.001 ***		<0.001 ***		
c.m.s.[a]	0.0124		0.0099		
$T_{0.05}$	1.96		2.00		

[a] The error mean squares (ems) are quoted in place of s.e.d. because of the variation in the number of observations (n) in week 8.

Table 6.16 : Mean speeds for each team in weeks 4 and 8 with ANOVA's to test for significant differences and week 8 speed expressed as a percentage of week 4.

Work output and energy expenditure

The mean work output of the control team was 2.13 ± 0.030 MJ/day (n = 10) with a range of 2.02 to 2.27 MJ/day. The mean estimated NE expenditure of the bulls for work (NE_{work}), was 130 ± 0.7 $kJ/kg^{0.75}$/day (n = 10), equivalent to 6.94 ± 0.065 MJ/head/day, or 37% of an animal's energy requirements for maintenance. The estimated NE_{work} of the other eight teams was very similar ranging from 127 to 140 $kJ/kg^{0.75}$/day (36 - 39% of the animals' maintenance energy requirements), with a mean of 134 ± 0.3 $kJ/kg^{0.75}$/day (n = 108).

Power output

The power output of the infected teams fell by an average of 30% within three weeks of the bulls becoming parasitaemic (Table 6.17), as lap times increased. The power output of the control team remained relatively constant over the same time period increasing slightly towards the end of the experiment as the bulls worked faster.

Week		4	4	5	6	7	8	9	10	11	12
Status		Uninfected	Uninf.	Pre-pat.	Parasit.	Parasit.	Parasit.	Parasit.	Parasit.	Parasit.	Parasit.
Group	Team	(Watts)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
HW	1	311	100	106	97	72	53	59			
HW	2	298	100	108	86	70	70				
HW	3	282	100	124	99	81	85	98	91	90	
HW	4	371	100	92	92	89	69	75	87	84	85
BW	5	319	100	92	74	60	51				
BW	6	324	100	105	98	66					
BW	7	340	100	103	100	84	81	86	90	91	93
BW	8	363	100	102	92	82	79	77	84	81	80
Control	9	330	100	103	102	100	96	102	103	107	108
HW	Mean	315.3 ± 19.44	100	108	93	78	69	78	89	87	85
BW	Mean	336.6 ± 9.74	100	101	91	73	71	81	87	86	87

Table 6.17 : Week 4 mean power outputs for each team, with outputs in subsequent weeks as percentages of output in week 4.

6.3.5 Feed intake

Rice bran & sesame cake

Rice bran intakes were very variable. For the first fortnight of the experiment all the animals consumed 100% of the bran offered, thereafter eight bulls spread across all groups except the control, consistently refused some or all of the bran. Once infected a further eight bulls began to leave some of their bran. From week 8 when sesame cake was mixed with the bran, hand feeding where necessary ensured 100% consumption by all animals. Individual weekly mean intakes are given in appendix 33.

Groundnut hay

Infection significantly depressed the groundnut hay consumption of the bulls, particularly those on the high plane of nutrition, HN and HW (Table 6.18). Prior to infection all animals consumed at least 99% of the hay offered but by the fourth week p.i. (week 8) the mean daily hay intake of the animals in HN had fallen by over 26% and that of the animals in HW by 9%. The mean hay intakes of groups BN and BW fell significantly between weeks 6 and 8, although the actual reductions in mean intake were small, no more than 2.5%. The mean intake of the animals in the control group also declined at this time. By week 9 the mean intakes of the basal and control groups had recovered but infected bulls on the high plane of nutrition, particularly those in HN, continued to refuse large quantities of their hay for the remainder of the experiment although intakes did improve. The mean intakes of HW p.i. were higher than those of HN, however the difference was only significant in weeks 6, 10, 11 and 12 (ANOVA, $F = 14.84$, 18.27, 9.66 and 15.92 respectively, $P < 0.01$, $n = 8$). Work had no significant effect on the intakes of the animals on the lower plane of nutrition. Mean daily hay intakes of individual bulls for each week of the experiment are given in appendix 34.

Mean DMI	BN		BW		HN		HW	
	T	P	T	P	T	P	T	P
Pre-inf. - week 5	1.89	0.18 ns	1.38	0.105 ns	2.91	0.011 *	0.70	0.25 ns
Pre-inf. - week 6	3.31	0.006 **	4.09	0.002 **	9.77	<0.001 ***	8.49	<0.001 ***
Pre-inf. - week 7	3.79	0.003 **	3.76	0.004 **	5.24	<0.001 ***	9.11	<0.001 ***
Pre-inf. - week 8	1.71	0.06 ns	2.43	0.023 *	5.17	<0.001 ***	8.30	<0.001 ***
Pre-inf. - week 9	1.00	0.18 ns	0.83	0.218 ns	5.65	<0.001 ***	4.15	0.002 **
Pre-inf. - week 10	1.00	0.18 ns	0.86	0.210 ns	10.85	<0.001 ***	4.63	0.001 **
Pre-inf. - week 11	1.00	0.18 ns	0.56	0.298 ns	9.44	<0.001 ***	3.90	0.003 **
Pre-inf. - week 12	1.00	0.18 ns	0.39	0.355 ns	9.99	<0.001 ***	3.30	0.007 **

Table 6.18 : Paired T- test results comparing individual animal mean groundnut intakes pre- and post-infection for each group (one-tailed tests of transformed data, n = 8).

Andropogon grass

Bulls on the basal plane of nutrition consumed significantly more andropogon than those on the high plane throughout the experiment, as expected (ANOVA, $F > 25$, $P < 0.001$, for all weeks of the experiment). Mean intakes of BN and BW were at least three times those of HN and HW respectively throughout the trial (Table 6.19). As with groundnut hay, trypanosome infection depressed andropogon intake. The intakes of the infected bulls fell significantly once they became parasitaemic (Table 6.20) and remained significantly below their pre-infection means until the end of the study. In contrast the mean intake of the control group rose slightly over the course of the experiment as illustrated in Figure 6.3. It was significantly higher than the mean intakes of HN and HW in week 3 and from weeks 6 to 12 inclusively (ANOVA, $F > 12$, $P \leq 0.01$).

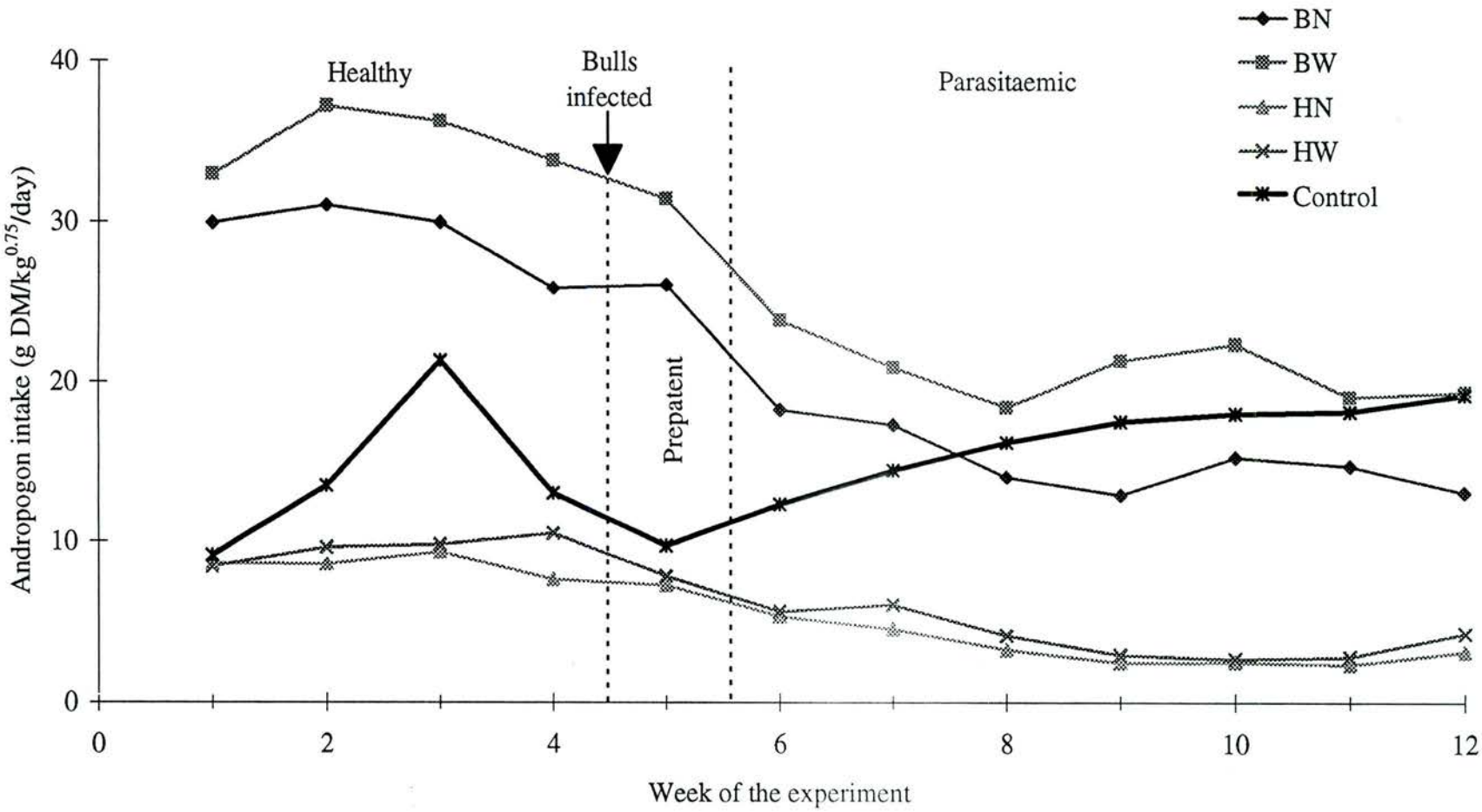
The mean andropogon intake of the working group on the basal diet (BW) was larger than that of the non-working group (BN) throughout the experiment (Figure 6.3), however the difference was only significant in weeks 4 and 9 (ANOVA, $F = 6.8$, $P = 0.02$ and $F = 5.5$, $P = 0.04$ respectively, $n = 8$). Work had no significant effect on the intakes of the infected bulls on the high plane of nutrition.

At times during the experiment andropogon intakes varied markedly between bulls within the same group, as indicated by the large coefficients of variation (c.v.) of some of the group means in Table 6.19. Some bulls consumed 2 - 3 times as much grass as their peers in the same group (individual andropogon intakes are given in appendix 35). The intake of bull 22 (HN) in particular was very different to that of its peers as illustrated in Figure 6.4. The data from this animal was considered so abnormal (no other bulls in the experiment showed such a pattern of grass consumption) that it was excluded from the HN dataset for statistical analysis.

Group	BN			BW			HN			HW			Control		
n	8			8			7			8			2		
Week	mean	s.e.	c.v.	mean	s.e.	c.v.	mean	s.e.	c.v.	mean	s.e.	c.v.	mean	s.e.	c.v.
1	29.9	2.38	21	32.9	3.20	27	8.6	0.56	17	8.4	0.62	21	9.1	0.38	6
2	31.0	1.86	17	37.2	3.07	23	8.6	1.35	42	9.6	1.04	30	13.5	2.72	28
3	30.0	2.01	19	36.3	2.54	20	9.3	1.32	37	9.8	1.51	44	21.4	3.63	24
4	25.9	1.55	17	33.8	2.61	22	7.6	1.09	38	10.5	1.22	33	13.1	0.88	9
5	26.1	2.14	23	31.5	2.15	19	7.2	1.13	41	7.8	1.16	42	9.7	1.29	19
6	18.3	1.89	29	23.9	2.36	28	5.3	0.61	31	5.6	0.64	33	12.3	1.18	14
7	17.4	1.33	22	20.9	1.99	27	4.5	0.71	42	6.0	0.93	44	14.5	3.28	32
8	14.1	1.30	26	18.4	2.49	38	3.2	0.34	28	4.1	0.65	45	16.2	3.84	33
9	13.0	1.15	25	21.4	3.42	45	2.4	0.26	28	2.9	0.38	38	17.5	4.47	36
10	15.3	1.30	24	22.4	3.41	43	2.5	0.34	36	2.7	0.34	36	18.1	2.97	23
11	14.8	1.35	26	19.1	4.06	60	2.3	0.20	22	2.8	0.34	34	18.2	2.01	16
12	13.1	1.17	25	19.4	4.43	64	3.1	0.47	40	4.3	0.87	58	19.3	0.18	1
Weeks 1 - 4	29.2	1.34	13	35.1	2.72	22	8.5	1.04	32	9.6	0.99	29	14.1	0.29	3
Weeks 6 - 12	15.1	1.18	22	20.8	3.00	41	3.4	0.35	28	4.1	0.44	30	16.6	2.57	22

Table 6.19 : Mean andropogon grass intakes for each group over the experiment with overall means for the uninfected (weeks 1 - 4) and parasitaemic (weeks 6 - 12) periods (g DM/kg$^{0.75}$/day) with standard errors and coefficients of variation (%).

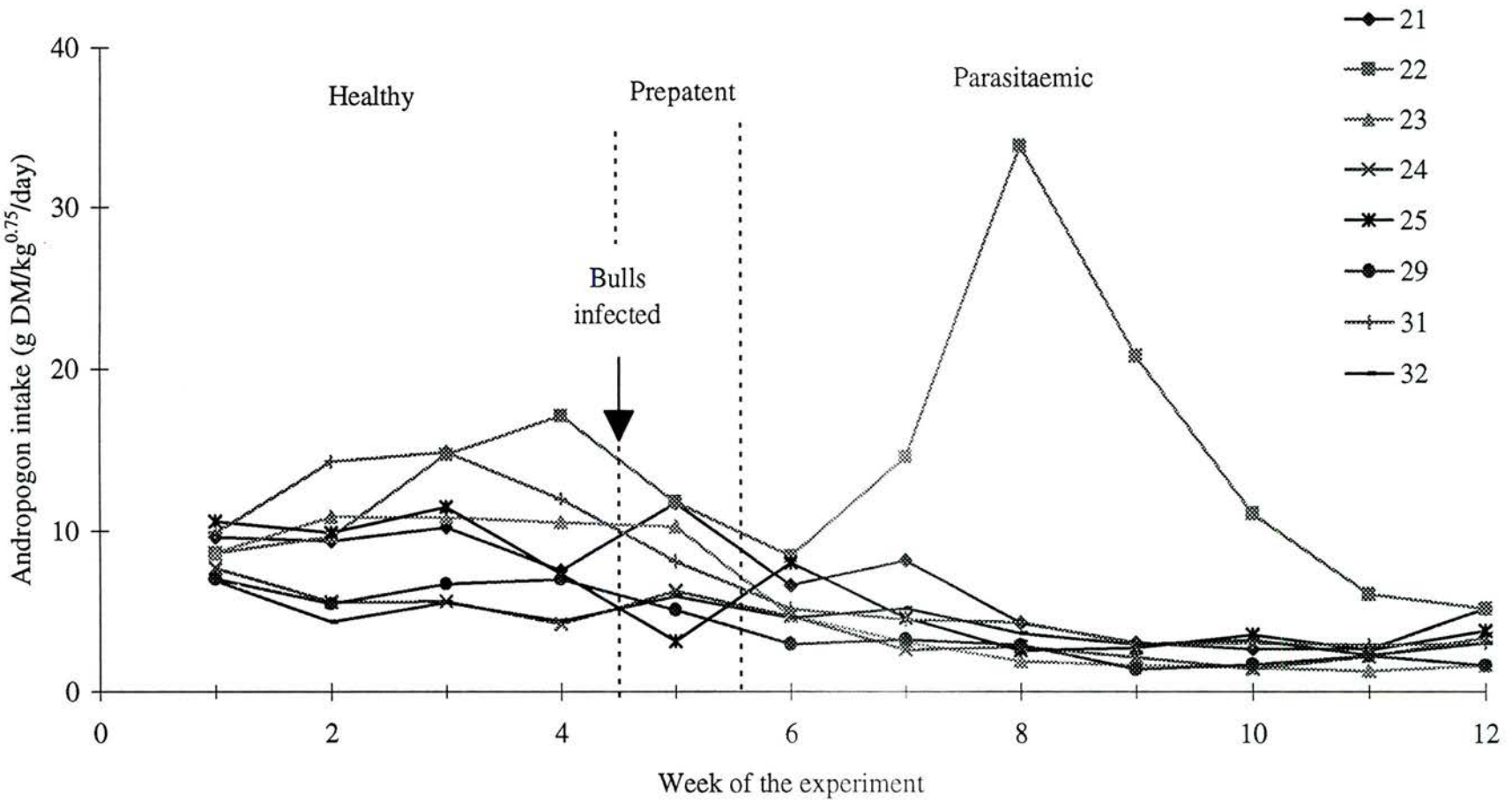
Figure 6.3 : Mean andropogon grass intakes for each group over the experiment.



Group	BN		BW		HN		HW	
(n)		8		8		7		8
Comparison	T	P	T	P	T	P	T	P
Pre-inf. - week 5	1.57	0.08 ns	4.71	0.001 **	1.05	0.168 ns	1.46	0.094 ns
Pre-inf. - week 6	7.76	<0.001 ***	7.21	<0.001 ***	3.42	0.007 **	3.17	0.008 **
Pre-inf. - week 7	7.74	<0.001 ***	8.06	<0.001 ***	3.51	<0.010 **	2.65	0.017 *
Pre-inf. - week 8	9.66	<0.001 ***	8.59	<0.001 ***	5.13	0.001 ***	4.05	0.002 **
Pre-inf. - week 9	12.62	<0.001 ***	4.96	<0.001 ***	6.09	<0.001 ***	6.36	<0.001 ***
Pre-inf. - week 10	15.63	<0.001 ***	4.55	<0.001 ***	6.11	<0.001 ***	6.91	<0.001 ***
Pre-inf. - week 11	8.90	<0.001 ***	5.48	<0.001 ***	6.10	<0.001 ***	5.98	<0.001 ***
Pre-inf. - week 12	16.56	<0.001 ***	5.29	<0.001 ***	4.91	0.001 ***	4.91	<0.001 ***

Table 6.20 : Paired T-test results comparing individual animal mean pre-infection andropogon grass intakes with intakes post-infection for each group to determine whether there was a significant fall in intake post-infection (one-tailed tests).

Figure 6.4 : Mean andropogon intakes for each HN bull for each week of the experiment.



Metabolisable energy intakes

Infection caused a significant fall in estimated ME intake regardless of plane of nutrition or level of exercise (Table 6.21), although amongst the bulls on the high plane of nutrition the effect was not significant until they became parasitaemic. This depression in ME intake was reversed in BN, BW and HW between weeks 7 and 8 when sesame cake was added to all rations (Figure 6.5), the exception being HN. ME intakes of bulls in this group p.i. remained significantly below their pre-infection means for all but two weeks of the parasitaemic period, despite the addition of sesame cake. The mean estimated ME intake of the two control bulls remained relatively constant with the exception of week 3, until the addition of the sesame when it increased by approximately 100 $kJ/kg^{0.75}$/day (Table 6.22).

Although the working groups had higher mean estimated ME intakes throughout the experiment than their non-working contemporaries on the same plane of nutrition, differences were not always significant (Table 6.23). The estimated ME intakes of the uninfected controls were significantly higher than those of the comparable infected group (HW) from week 6 onwards when the HW animals developed patent parasitaemias. The weekly mean estimated ME intakes for each bull are given in appendix 36.

Group	BN		BW		HN		HW	
(n)	8		8		7		8	
Comparison	F	P	F	P	F	P	F	P
Pre-inf. - week 5	1.93	0.047 *	6.48	< 0.001 ***	1.36	0.112 ns	1.35	0.110 ns
Pre-inf. - week 6	10.03	<0.001 ***	8.22	<0.001 ***	6.48	<0.001 ***	6.24	<0.001 ***
Pre-inf. - week 7	10.17	<0.001 ***	8.60	<0.001 ***	5.52	<0.001 ***	5.54	<0.001 ***
Pre-inf. - week 8	1.21	0.132 ns	0.92	0.194 ns	3.64	0.005 **	0.57	0.294 ns
Pre-inf. - week 9	1.68	0.068 ns	0.45	0.332 ns[a]	3.02	0.012 *	0.07	0.474 ns[a]
Pre-inf. - week 10	1.27	0.123 ns[a]	1.48	0.091 ns[a]	0.98	0.181 ns	4.14	0.002 ** [a]
Pre-inf. - week 11	0.18	0.431 ns	0.12	0.453 ns	2.45	0.025 *	1.22	0.130 ns[a]
Pre-inf. - week 12	1.90	0.050 ns	0.01	0.494 ns	1.63	0.077 ns	3.25	0.007 ** [a]

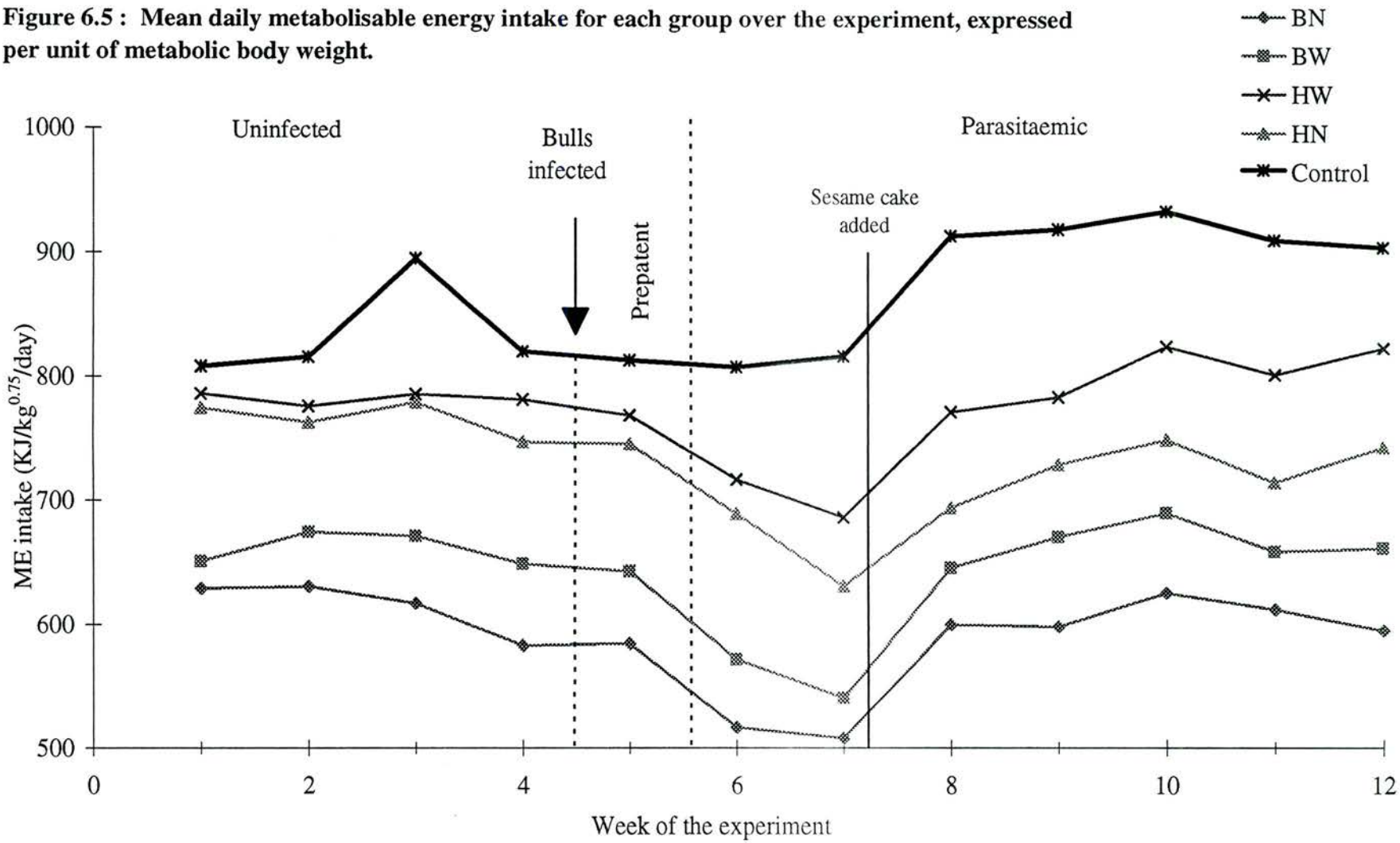
[a] Intake p.i. larger than pre-infection intake.

Table 6.21 : Paired T- test results comparing individual animal mean pre-infection metabolisable energy intakes with intakes post-infection for each group to determine whether there was a significant fall in intake after infection (one-tailed tests).

Group	BN		BW		HN		HW		Control	
(n)	8		8		7		8		2	
Week	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
1	629	16.6	650	22.5	774	11.4	786	7.4	808	17.3
2	630	13.2	674	19.9	763	14.9	776	7.7	816	43.7
3	616	17.9	671	20.3	779	13.7	785	8.2	895	7.2
4	583	17.3	648	22.1	747	14.2	781	5.3	820	29.2
5	585	19.0	643	18.7	746	15.6	769	10.4	813	30.6
6	517	15.0	572	20.7	689	7.2	717	9.2	807	14.3
7	508	8.9	540	12.3	631	18.2	686	14.9	816	2.9
8	600	5.9	645	20.1	694	15.4	772	16.1	914	4.8
9	598	7.8	670	25.6	729	12.0	783	14.7	919	6.2
10	625	8.3	690	22.7	749	9.8	825	9.6	934	8.2
11	612	10.1	659	24.7	715	15.4	802	17.0	911	19.5
12	595	8.1	661	26.5	743	9.7	823	14.0	905	31.9
Weeks 1 - 4	614	10.7	661	20.3	766	13.0	782	5.4	835	20.8
Weeks 6 - 12	579	7.2	634	20.3	707	10.8	773	9.1	887	11.2

Table 6.22 : Mean ME intakes per unit of metabolic liveweight for each group over the study with overall means for the uninfected (weeks 1 - 4) and the parasitaemic (weeks 6 - 12) periods (kJ /kg$^{0.75}$/day).

Figure 6.5 : Mean daily metabolisable energy intake for each group over the experiment, expressed per unit of metabolic body weight.



Week	BN versus BW		HN versus HW		HW versus Control	
	F	P	F	P	F	P
1 to 4 Uninf.	4.1	0.062 ns	1.5	0.243 ns	14.3	0.005 **
5	4.7	0.048 *	1.6	0.231 ns	3.1	0.116 ns
6	4.6	0.049 *	5.4	0.037 *	20.3	< 0.001 ***
7	4.7	0.049 *	5.8	0.032 *	17.3	0.003 **
8	4.7	0.047 *	11.9	0.004 **	17.9	0.003 **
9	7.3	0.017 *	7.9	0.015 *	19.5	0.002 **
10	7.2	0.018 *	30.2	< 0.001 ***	28.6	< 0.001 ***
11	3.1	0.100 ns	14.1	0.002 **	9.0	0.017 *
12	5.8	0.031 *	21.1	< 0.001 ***	6.5	0.034 *

Table 6.23 : ANOVA's of ME intakes within planes of nutrition over the experiment.

6.3.6 Body temperatures

Infection caused a significant rise in the 08.00 h body temperatures of all bulls and a significant rise in 12.00 h body temperatures of all except BN 8. Pre-infection 08.00 h temperatures (period 1) ranged from 34.2 to 38.7°C, once parasitaemic (period 3) the range was 35.1 to 40.1°C. In contrast the 08.00 h temperatures of the two control animals were significantly lower in period 3 than they had been in period 1 (MW, $W > 700$, $P < 0.01$ for both animals, $n_1 = 20$, $n_2 = 33$). The 12.00 h temperatures of these two animals did not vary between periods (MW, $W \leq 83$, $P > 0.05$, $n_1 = 20$, $n_2 = 33$). Median body temperatures for each bull at 08.00 and 12.00 h in each period are given in appendix 37.

The body temperatures of all animals increased between 08.00 and 12.00 h each day as the air temperature rose, irrespective of whether they were worked.

Work did exacerbate the increase. The median temperatures of groups BW and HW at 12.00 h after work, were significantly higher than those of the two non-working groups in each period (Table 6.24). The median temperature of HN at 08.00 h was significantly higher than that of BN throughout the experiment, HW was significantly higher than BN once the bulls were parasitaemic. The control group median temperatures at 08.00 and 12.00 h were higher than most other groups throughout the experiment however these results being derived from only two replicates were excluded from the statistical analysis (Table 6.24).

6.3.7 Meteorology

It was significantly hotter at 08.00 h and more humid at 08.00 and 15.00 h during period 3 of the experiment than during either of the two previous periods (Table 6.25). The temperature at 15.00 h did not vary significantly between periods. The minimum temperature recorded during a working day was 15°C at 08.00 h, the maximum 41°C at 15.00 h, relative humidity varied from 29 to 100%. No rain fell during the experiment. Mean monthly temperatures and relative humidities during the experiment are given in Table 6.26.

Period	Uninfected	Uninfected	Pre-patent	Pre-patent	Parasitaemic	Parasitaemic	(n)
Time	08.00 h	12.00 h	08.00 h	12.00 h	08.00 h	12.00 h	
Group	median & s.i.r.	median & s.i.r.	median & s.i.r.	median & s.i.r.	median & s.i.r.	median & s.i.r.	
BN	36.6 0.12	38.0 0.18	36.3 0.21	37.6 0.27	37.3 0.23	38.6 0.38	8
BW	36.8 0.17	38.9 0.09	36.6 0.29	39.1 0.15	37.6 0.30	39.7 0.16	8
HN	37.0 0.24	37.9 0.20	36.9 0.30	37.8 0.19	37.7 0.18	38.9 0.20	8
HW	36.9 0.25	38.9 0.20	36.6 0.29	39.0 0.22	38.0 0.35	39.7 0.28	8
H	12.20	23.29	8.17	23.62	11.58	19.16	
P_{group}	0.007 **	< 0.001 ***	0.043 *	< 0.001 ***	0.009 **	< 0.001 ***	
Control	37.4	39.2	37.1	39.6	37.0	39.3	2

Table 6.24 : Median body temperatures with s.i.r for each of the infected groups at 08.00 and 12.00 h for each period of the study with results of Kruskal Wallis tests to look for significant differences.

(Medians for the control group were not included in the analysis because of the small sample size and are presented for information only.)

Period of the experiment	Diurnal temperature change (°C)	Temperature at 08.00 h (°C)	Rel. humidity at 08.00 h (%)		Temperature at 15.00 h (°C)	Rel. humidity at 15.00 h (%)	
	mean ± s.e.	mean ± s.e.	mean ± s.e.	(n)	mean ± s.e.	mean ± s.e.	(n)
Uninfected (1)	15.5 ± 0.67	18.1 ± 0.35	58 ± 2.9	26	30.4 ± 1.20	36 ± 3.6	13
Pre-patent (2)	17.9 ± 1.61	18.3 ± 0.41	61 ± 5.9	9	34.0 ± 1.60	29 ± 5.9	7
Parasitaemic (3)	12.6 ± 0.71	20.4 ± 0.24	76 ± 1.7	47	30.1 ± 0.71	50 ± 2.5	38
F	7.15	18.73	16.03		2.38	8.84	
P_{period}	0.001 ***	<0.001 ***	<0.001 ***	n_1, n_2	0.102 ns	<0.001 ***	n_1, n_2
s.e.d.	1.09	0.41	3.31	47, 26	1.39	4.76	38, 13
s.e.d.	1.62	0.61	4.92	47, 9	1.78	6.09	38, 7
s.e.d.	1.72	0.65	5.23	26, 9	2.03	6.94	13, 7

Table 6.25 : Mean temperatures and relative humidities for each period of the experiment, with ANOVA results.

Month	Day of the experiment	Diurnal temp. change (°C)	Temperature at 08.00 h (°C)	Rel. humidity at 08.00 h (%)		Temperature at 15.00 h (°C)	Rel. humidity at 15.00 h (%)	
		mean ± s.e.	mean ± s.e.	mean ± s.e.	(n)	mean ± s.e.	mean ± s.e.	(n)
JAN	1 - 22	15.5 ± 0.6	17.9 ± 0.28	60 ± 2.1	31	29.4 ± 1.42	37 ± 4.1	9
FEB	23 - 50	16.2 ± 0.86	19.1 ± 0.26	65 ± 3.3	28	32.1 ± 0.89	38 ± 3.4	25
MAR	51 - 81	11.3 ± 0.78	20.9 ± 0.28	77 ± 2.1	31	29.6 ± 0.87	53 ± 2.8	24

Table 6.26 : Monthly mean temperatures and humidity levels during the experiment.

6.3.8 Parasitaemias

Working the bulls significantly increased their parasitaemias (Table 6.27), plane of nutrition had no effect on parasitaemia. The two non-working groups had lower peak parasitaemia scores, fewer days with scores of 5+ or higher and lower mean scores once infected than the working groups (Table 6.28). BN had a significantly smaller mean peak score than BW, fewer days with scores of 5+ or higher than either BW or HW and a lower mean parasitaemia score than HW. Figure 6.6 shows the mean parasitaemia score of each group on each sampling occasion p.i.. The mean score of BN is below those of the other three groups for the majority of the parasitaemic period. The rate of decline of parasitaemia post peak score did not differ significantly between groups (Table 6.28).

Parasites were detected in blood samples (by MHCT) from all infected animals within nine days of infection. Some animals became parasitaemic as early as 3 - 4 days p.i.. There was however no significant difference between the groups in the number of trypanosome negative (by MHCT) blood samples obtained from each bull before trypanosomes were first detected (Table 6.28). The exact duration of prepatency could not be consistently determined, because of the irregular sampling intervals used (blood samples were collected on Mondays, Wednesdays and Fridays). Only 6 out of 672 blood samples were trypanosome negative by MHCT after prepatency, these 6 samples came from 4 different animals. Peak parasitaemia occurred in most bulls within 13 - 16 days of infection with subsequent decreasing waves of parasitaemia at approximately 10 day intervals. All the bulls remained parasitaemic (by MHCT) at the end of the experiment, none of them having self-cured.

Removing the parasitaemia scores of animals 19 and 28 (HW) which had proved agglutination test positive prior to the start of the experiment had no effect on the results. Data from these two animals was therefore retained in the analyses.

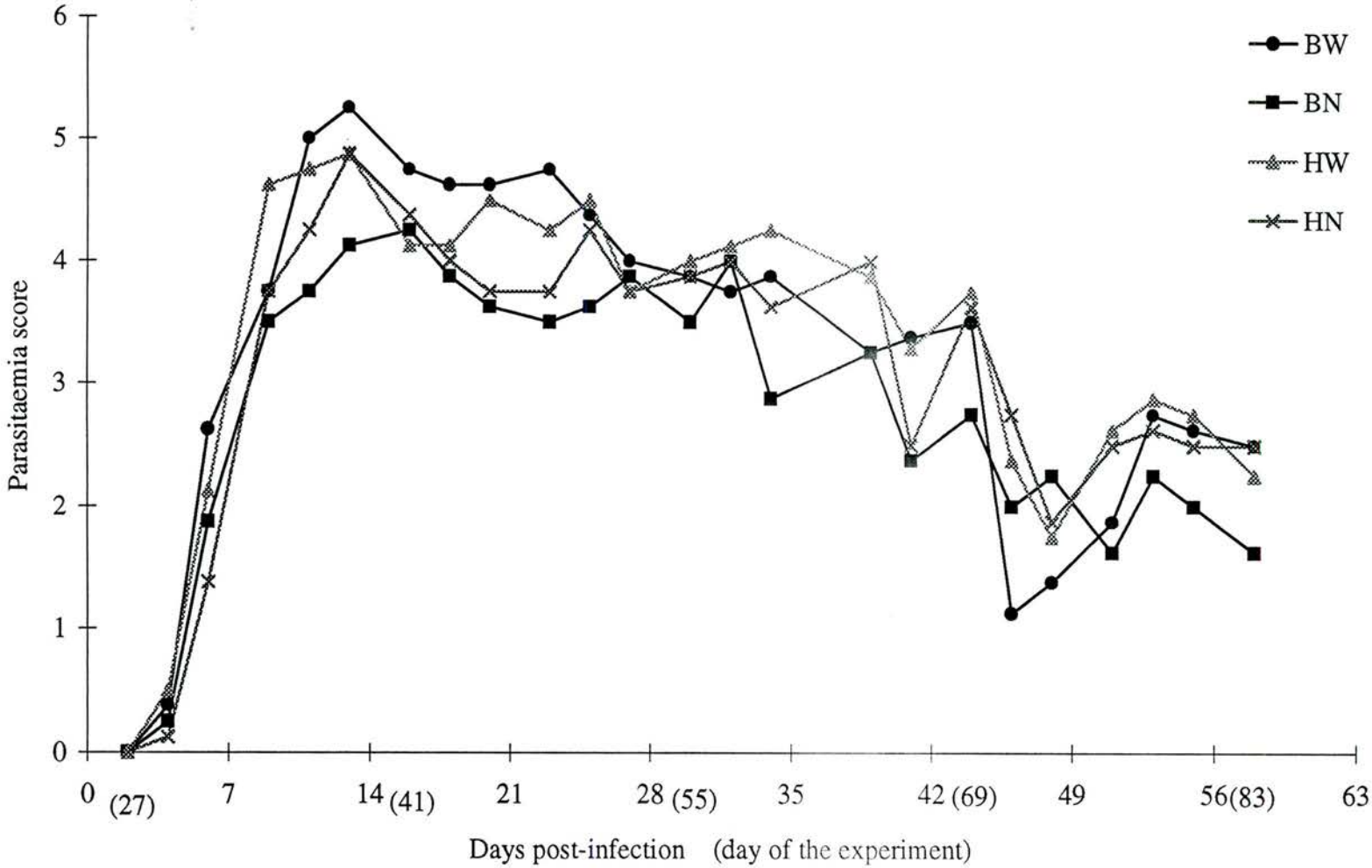
Parameter	Basal diet		High diet		F	$P_{diet.}$	No work		Work		F	P_{work}	F	$P_{diet.x\ work}$
ANOVA	**mean**	**s.e.**	**mean**	**s.e.**			mean	s.e.	mean	s.e.				
Number of prepatent samples	1.8	0.10	1.9	0.16	0.1	0.74 ns	1.9	0.11	1.8	0.14	1.0	0.33 ns	0.1	0.74 ns
Peak parasitaemia score	5.2	0.19	5.3	0.14	0.1	0.77 ns	4.9	0.14	5.5	0.16	7.0	0.01 **	2.2	0.15 ns
Days with scores of ≥ 5+	4.3	0.82	5.0	0.67	0.5	0.47 ns	3.4	0.71	5.9	0.65	6.8	0.02 *	3.2	0.08 ns
Rate of decline of parasit. post peak score (score /day)	0.07	0.005	0.05	0.006	3.7	0.07 ns	0.06	0.005	0.06	0.006	1.0	0.33 ns	0.6	0.45 ns
Mann Whitney test	**median**	**s.i.r.**	**median**	**s.i.r.**	**W**	$P_{diet.}$	median	s.i.r.	median	s.i.r.	W	P_{work}		
Bull mean scores once parasit.	3.4	0.40	3.6	0.12	227	0.17 ns	3.3	0.36	3.6	0.08	192	0.003***		

Table 6.27 : Dietary and work effects on parasitaemia parameters with ANOVA and Mann-Whitney test (MW) results (n = 16).

Parameter	BN		BW		HN		HW		F	P_{group}	s.e.d.
ANOVA	**mean**	**s.e.**	**mean**	**s.e.**	mean	s.e.	**mean**	**s.e.**			
Number of prepatent samples	1.9	0.13	1.8	0.16	2.0	0.19	1.8	0.25	0.41	0.75 ns	
Peak parasitaemia score	4.8	0.16	5.6	0.26	5.1	0.23	5.4	0.18	3.08	0.04 *	0.30
Days with scores of 5+ or higher	2.3	0.65	6.4	1.12	4.6	1.16	5.4	0.71	3.51	0.03 *	1.33
Rate of decline of parasitaemia post peak score (score/day)	0.06	0.009	0.07	0.005	0.05	0.007	0.05	0.009	1.75	0.18 ns	
Kruskal Wallis test	**median**	**s.i.r.**	**median**	**s.i.r.**	median	s.i.r.	**median**	**s.i.r.**	**W**	P_{group}	
Bull mean scores once parasitaemic	3.0	0.36	3.6	0.15	3.4	0.32	3.6	0.06	10.4	0.02 *	

Table 6.28 : Group mean parasite parameters with ANOVA and Kruskal-Wallis (KW) test results (n = 8).

Figure 6.6 : Mean parasitaemia scores for each group p.i.



6.3.9 Haematology

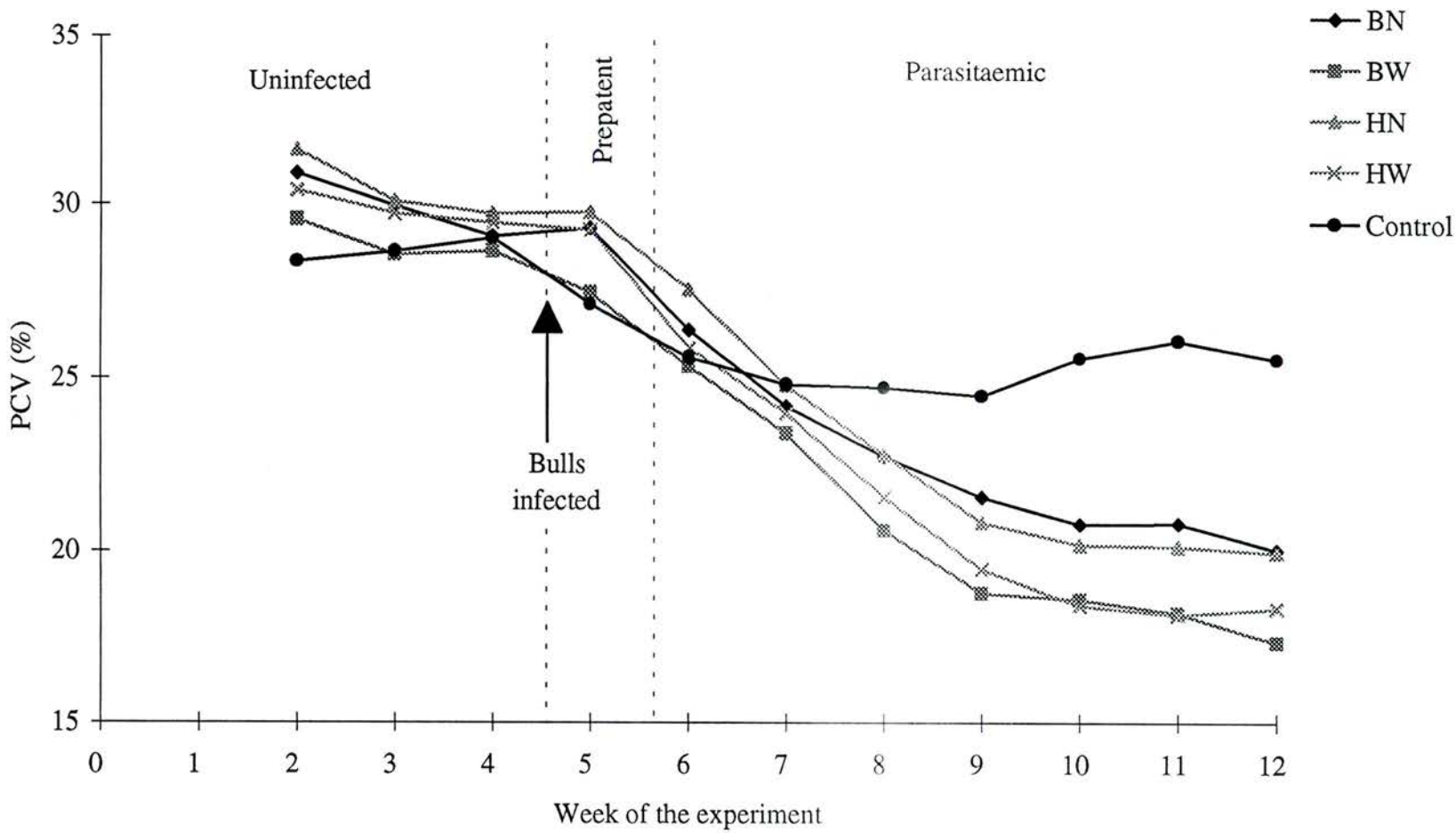
Packed cell volume

Trypanosomosis caused a marked fall in PCV, which was exacerbated by work but not by plane of nutrition. The PCV's of all bulls fell over the course of the experiment. The decline was only slight in the uninfected controls, but much more pronounced in the 32 infected animals p.i., particularly those that were worked (Figure 6.7). In week 2 the mean PCV of all 34 bulls was 30.5 ± 0.49%. By week 12 the mean of the 32 infected animals had fallen to 18.9 ± 0.47%, compared to 25.5 ± 0.50% for the two controls.

The mean PCV's of the five groups did not differ significantly prior to infection nor in week 6 when the bulls first became parasitaemic (Table 6.29). However by week 9 the control mean was higher than that of either of the infected working groups, but not significantly different to those of the two non-working infected groups. By the end of the experiment the mean PCV of the control bulls was significantly higher than that of any of the infected groups, and the means of the non-working groups were higher than those of the working groups (Table 6.29). The PCV's of the bulls in the infected groups declined more rapidly than those of the uninfected controls over weeks 5 to 9. The effects of work and nutrition on PCV are detailed in Table 6.30. Weekly mean PCV's for all animals are given in appendix 38

Amongst the infected animals there was a strong positive linear correlation between PCV at the start of the experiment and PCV at the end ($R = 0.504$, $P < 0.01$, $n = 32$). Bulls starting with higher than average PCV's tended to maintain relatively high PCV's compared to their contemporaries once infected and vice versa (Figure 6.8). Furthermore bulls which developed high mean parasitaemias once infected had lower mean PCV's over the same period ($R = 0.372$, $P = 0.036$, $n= 32$).

Figure 6.7 : Mean PCV for each group for each week of the experiment.



Group	BN (%)		BW (%)		HN (%)		HW (%)		Control (%)		F	P_{group}	s.e.d. (n_1=2,	s.e.d. (n_1=8,
Week	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.			n_2=8)	n_2=8)
2	30.92	0.620	29.56	1.209	31.61	1.177	30.42	0.978	28.35	0.983	0.85	0.50 ns		
4	29.09	0.744	28.65	1.150	29.73	1.262	29.47	1.140	29.03	0.433	0.15	0.96 ns		
6	26.38	0.643	25.33	0.715	27.54	0.910	25.88	0.972	25.58	2.020	1.01	0.42 ns		
9	21.55	0.649	18.77	0.948	20.82	0.869	19.45	1.070	24.48	0.783	2.96	0.04 *	1.98	1.25
12	20.01	0.928	17.31	0.969	19.94	0.920	18.30	0.687	25.53	0.500	5.15	0.003 **	1.94	1.23
Rate$^+$	0.27	0.025	0.32	0.038	0.32	0.023	0.34	0.028	0.09	0.017	4.39	0.007 **	0.131	0.083
(n)	8		8		8		8		2					

Rate$^+$ Rate of decline of PCV between weeks 5 and 9 (% /d)

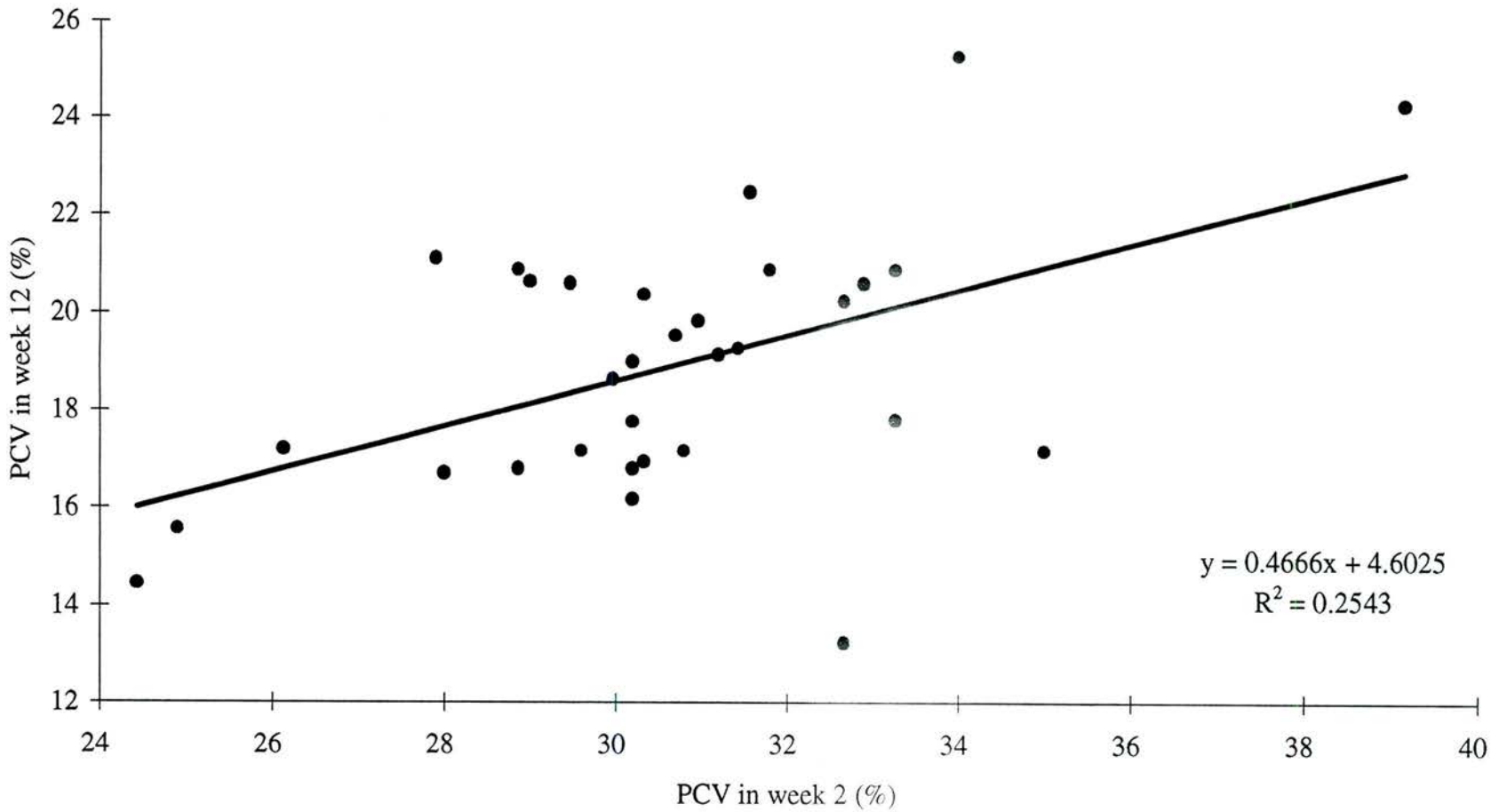
Table 6.29 : Group mean PCV's at specific points during the study, with ANOVA results indicating significant differences.

Week	Basal diet (%) mean	s.e.	High diet (%) mean	s.e.	F	P_{diet}	No work (%) mean	s.e.	Work (%) mean	s.e.	F	P_{work}	F	$P_{diet \times work}$
2	30.24	0.679	31.01	0.755	0.57	0.46 ns	31.26	0.649	29.99	0.759	1.55	0.22 ns	0.94	0.94 ns
4	28.87	0.664	29.60	0.821	0.45	0.51 ns	29.41	0.712	29.06	0.788	0.10	0.75 ns	0.01	0.93 ns
6	25.85	0.484	26.71	0.678	1.09	0.31 ns	26.96	0.559	25.60	0.587	2.73	0.11 ns	0.14	0.71 ns
9	20.16	0.661	20.14	0.687	0.00	0.98 ns	21.18	0.532	19.11	0.695	5.36	0.03 *	0.62	0.44 ns
12	18.66	0.736	19.12	0.593	0.27	0.61 ns	19.97	0.631	17.81	0.588	6.01	0.02 *	0.36	0.55 ns
Rate^{+}	0.30	0.023	0.33	0.018	1.67	0.21 ns	0.30	0.018	0.33	0.023	1.04	0.32 ns	0.16	0.69 ns

Rate^{+} Rate of decline of PCV between weeks 5 and 9 (% /d)

Table 6.30 : Dietary and work effects on PCV at specific points during the study, with ANOVA results indicating significant differences (n=16).

Figure 6.8 : PCV's of the 32 infected bulls in week 2 compared to their PCV's in week 12.



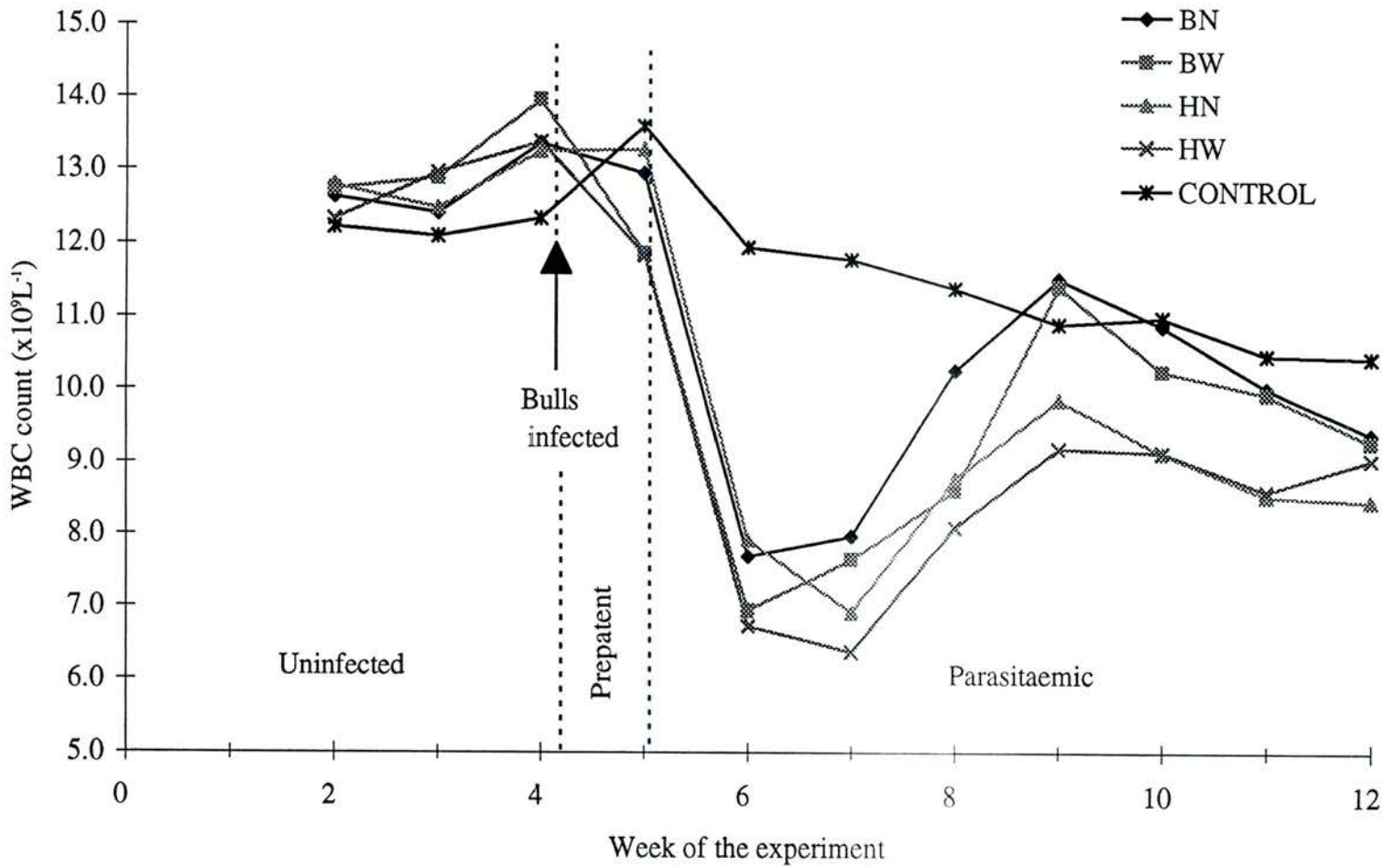
White blood cell counts

The WBC counts of the infected bulls fell by 40 - 50% as the animals became parasitaemic. They then recovered slightly during weeks 8 and 9 before falling once more, albeit much more slowly over the last three weeks of the experiment (Figure 6.9). The WBC counts of the uninfected controls declined only slightly over the experiment.

There were no significant differences in the median WBC counts of the groups in weeks 2, 4 and 6, however in weeks 6 and 7 the mean counts of the control group were significantly higher than those of the infected groups (Table 6.31) as might be expected from Figure 6.9. In weeks 2 and 4 the data was not normally distributed hence KW tests were used for analysis. In week 6 the data was almost normally distributed (Shapiro-Wilks test, $P = 0.08$) and both non-parametric and more powerful parametric tests were used. From week 7 onwards ANOVA's were appropriate.

Plane of nutrition had a transient effect on WBC count. In week 9 bulls on the high plane of nutrition had significantly higher counts than those on the low plane (Table 6.32). There were no other dietary nor work effects nor any interactions. Weekly mean WBC counts for individual animals over the course of the study are given in appendix 39.

Figure 6.9: Mean white blood cell (WBC) counts for each group over the experiment.



Week	BN median	BN s.i.r	BW median	BW s.i.r	HN median	HN s.i.r	HW median	HW s.i.r	Control median	Control s.i.r	KW test H	P_{group}	s.e.d. ($n_1 = 2, n_2 = 8$)	s.e.d. ($n_1 = 8, n_2 = 8$)
2	12.3	2.32	12.3	1.71	12.5	1.52	12.0	1.73	12.2	–	0.28	0.99 ns		
4	12.9	2.65	13.5	2.11	12.6	1.43	12.2	2.42	12.3	–	0.72	0.95 ns		
6	7.8	1.25	6.5	1.20	7.1	1.31	7.0	1.01	12.0	–	7.93	0.10 ns		
	mean	**s.e.**	**mean**	**s.e.**	**mean**	**s.e.**	**mean**	**s.e.**	mean	s.e.	**ANOVA**			
2	12.6	0.88	12.7	0.76	12.8	1.01	12.3	0.72	12.2	0.62	not valid			
4	13.4	1.12	14.0	0.86	13.3	0.94	13.4	1.04	12.3	0.64	not valid			
6	7.7	0.48	6.9	0.59	7.9	0.60	6.7	0.38	12.0	0.72	5.72	0.002 **	1.15	0.73
7	8.0	0.51	7.7	0.76	6.9	0.68	6.4	0.29	11.8	1.28	4.61	0.005 **	1.33	0.84
9	11.5	0.74	11.4	0.82	9.9	0.98	9.2	0.55	10.9	0.39	1.71	0.18 ns		
12	9.4	0.83	9.3	0.87	8.5	0.90	9.1	0.64	10.5	0.62	0.38	0.82 ns		
(n)	8		8		8		8		2					

Table 6.31 : Group mean WBC counts ($1 \times 10^9 L^{-1}$) at specific points during the study, with statistical analysis of the means.

Week	Basal diet mean	Basal diet s.e.	High diet mean	High diet s.e.	F	P_{diet}	No work mean	No work s.e.	Work mean	Work s.e.	F	P_{work}	F	$P_{diet \times work}$
6	7.3	0.38	7.3	0.37	0.00	0.99 ns	7.8	0.37	6.8	0.34	3.52	0.07 ns	0.18	0.67 ns
7	7.8	0.45	6.6	0.34	3.93	0.06 ns	7.5	0.43	7.0	0.43	0.55	0.47 ns	0.03	0.86 ns
9	11.5	0.53	9.5	0.55	6.19	0.02 *	10.7	0.63	10.3	0.56	0.24	0.63 ns	0.12	0.73 ns
12	9.3	0.58	8.8	0.54	0.50	0.48 ns	8.9	0.60	9.2	0.52	0.09	0.77 ns	0.17	0.68 ns

Table 6.32 : Dietary and work effects on WBC counts($1 \times 10^9 L^{-1}$) at specific points during the study, ANOVA results (n = 16).

Red blood cell counts

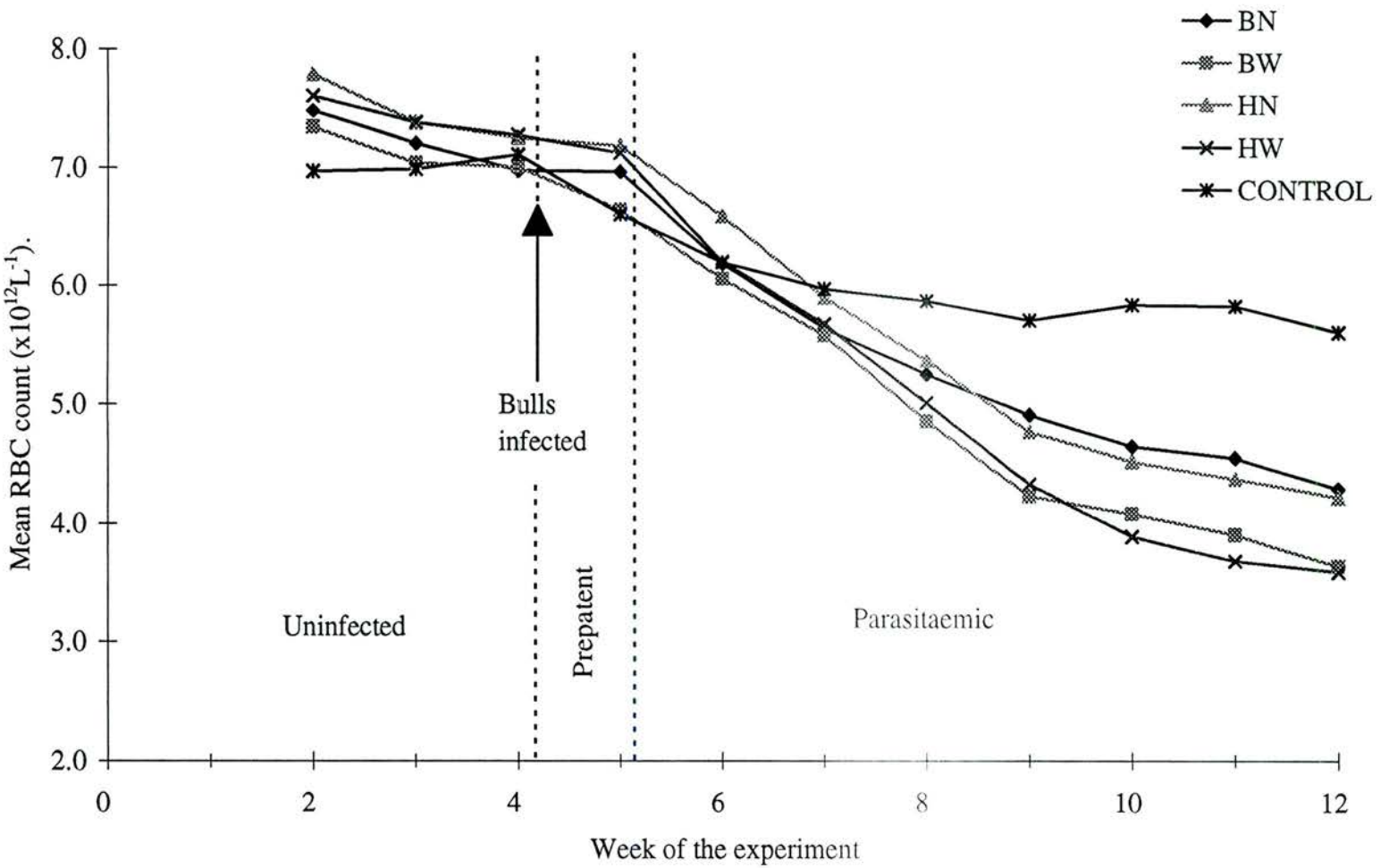
The RBC counts of the infected bulls fell markedly over the course of the experiment (Figure 6.10), in some cases to less than 50% of week 2 values, mirroring the decline in PCV. The counts of the control bulls declined only slightly from weeks 2 - 12, although the differences between the mean count of the uninfected controls and those of the infected groups did not become significant until week 10. In weeks 10 and 12 the control means were significantly higher than those of the 4 infected groups (Table 6.33).

Work significantly reduced the mean RBC counts of the infected bulls from week 9 to the end of the experiment (Table 6.34). Plane of nutrition did not significantly affect RBC count, nor were there any interactions between nutrition and work. Weekly mean RBC counts for each bull throughout the study are given in appendix 40.

Mean red cell volume

Mean red cell volumes (MCV) increased slightly in all bulls over the course of the experiment as PCV's and RBC's fell. The MCV of the two control bulls rose from an average of 41 ± 2.0 fl in week 2 to 46 ± 2.3 fl in week 12, and in the infected animals from 41 ± 0.4 fl to 49 ± 0.77 fl.

Figure 6.10: Mean red blood cell (RBC) count for each group over the study.



Group	BN		BW		HN		HW		Control		F	P_{group}	s.e.d.	s.e.d.
Week	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.			($n_1 = 2, n_2 = 8$)	($n_1 = 8, n_2 = 8$)
2	7.5	0.29	7.3	0.39	7.8	0.26	7.6	0.29	7.0	0.92	0.47	0.76 ns		
4	7.0	0.30	7.0	0.37	7.3	0.28	7.3	0.27	7.1	0.79	0.19	0.94 ns		
6	6.2	0.25	6.1	0.26	6.6	0.21	6.2	0.21	6.2	1.05	0.64	0.64 ns		
9	4.9	0.19	4.2	0.35	4.8	0.20	4.3	0.27	5.7	0.80	2.25	0.09 ns		
10	4.7	0.18	4.1	0.39	4.5	0.15	3.9	0.24	5.9	0.76	3.51	0.02 *	0.58	0.37
12	4.3	0.26	3.7	0.37	4.2	0.21	3.6	0.19	5.6	0.47	3.78	0.01 **	0.60	0.38
(n)	8		8		8		8		2					

Table 6.33 : Group mean RBC counts ($1 \times 10^{12}L^{-1}$) at specific points during the experiment, with ANOVA results.

	Basal diet		High diet		F	P_{diet}	No work		Work		F	P_{work}	F	$P_{diet \times work}$
Week	mean	s.e.	mean	s.e.			mean	s.e.	mean	s.e.				
2	7.4	0.24	7.7	0.19	0.86	0.36 ns	7.6	0.19	7.5	0.23	0.27	0.61 ns	0.01	0.94 ns
4	7.0	0.23	7.3	0.19	0.77	0.39 ns	7.1	0.20	7.1	0.22	0.01	0.94 ns	0.00	0.99 ns
6	6.1	0.17	6.4	0.15	1.38	0.25 ns	6.4	0.16	6.1	0.16	1.24	0.27 ns	0.31	0.58 ns
9	4.6	0.21	4.6	0.17	0.01	0.93 ns	4.8	0.13	4.3	0.21	4.63	0.04 *	0.21	0.65 ns
12	4.0	0.24	3.9	0.16	0.05	0.83 ns	4.3	0.16	3.6	0.20	5.71	0.02 *	0.00	0.97 ns

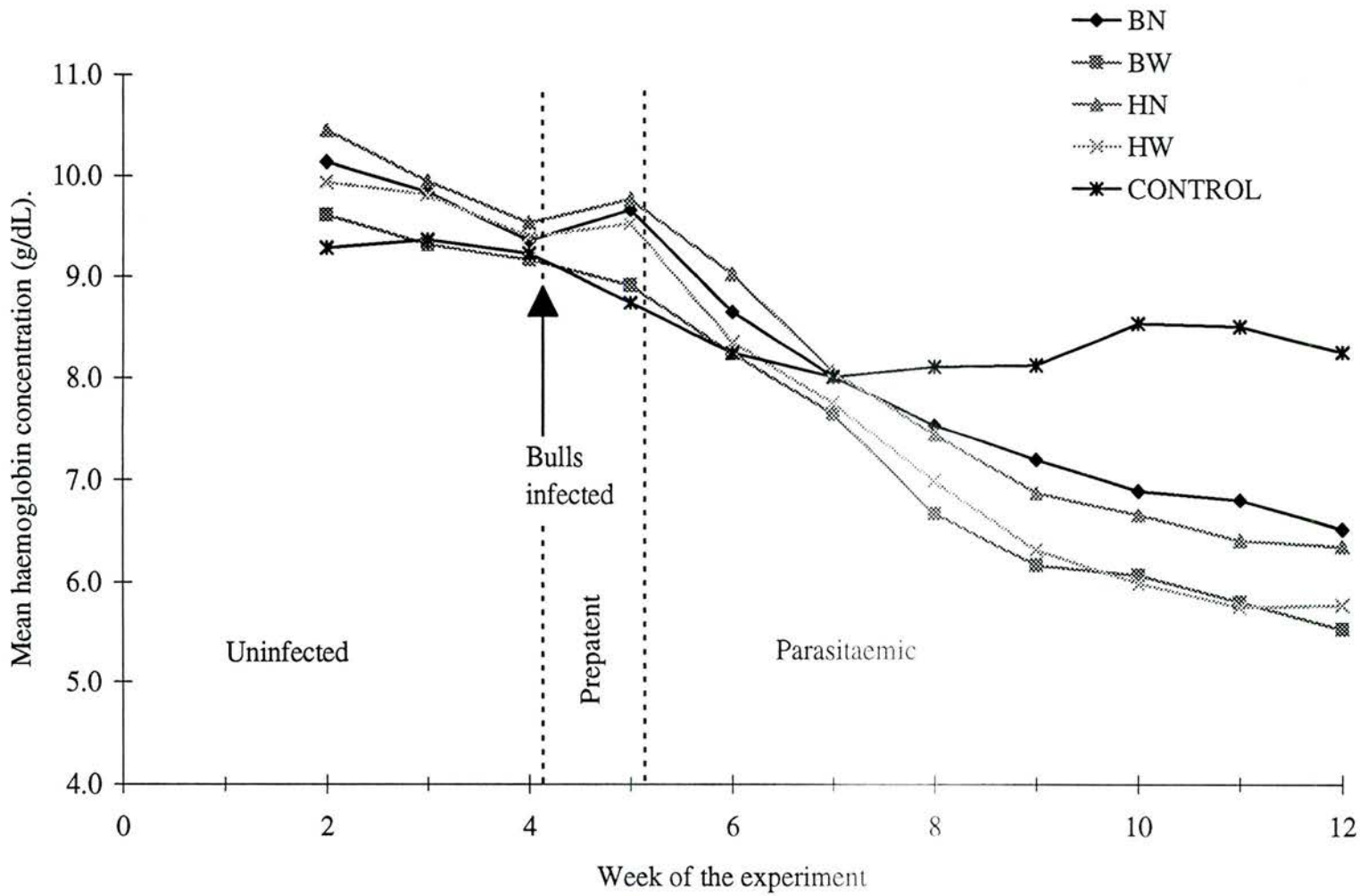
Table 6.34 : Dietary and work effects on RBC counts ($1 \times 10^{12}L^{-1}$) at specific points during the study, with ANOVA's to determine significant differences (n = 16).

Haemoglobin

Infection caused a decline in blood haemoglobin concentrations similar to that observed in both PCV's and RBC counts. Changes in the mean haemoglobin concentrations of each group are illustrated in Figure 6.11. From week 9 onwards the mean haemoglobin concentration of the uninfected control group was significantly higher than those of either of the working groups of bulls (Table 6.35). By week 10 it was higher than those of all four of the infected groups. Work significantly reduced the haemoglobin concentrations of the infected bulls in the last 4 weeks of the experiment (Table 6.36). In contrast diet had no significant effect on haemoglobin levels. Weekly mean haemoglobin levels for individual bulls are detailed in appendix 41.

Mean corpuscular haemoglobin (MCH), increased slightly in all bulls over the course of the experiment, from a mean of 13.3 ± 0.16 pg in week 2 to 15.5 ± 0.24 pg by week 12 (n =34), (Figure 6.12). Mean corpuscular haemoglobin concentrations (MCHC), remained almost constant, 32.7 ± 0.13 g/dl in week 2 compared to 32.0 ± 0.16 g/dl in the final week (n = 34), (Figure 6.13).

Figure 6.11: Mean haemoglobin concentrations for each group over the study.



Group	BN		BW		HN		HW		Control		F	P_{group}	s.e.d.	s.e.d.
Week	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.			($n_1 = 2, n_2 = 8$)	($n_1 = 8, n_2 = 8$)
2	10.1	0.21	9.6	0.50	10. 5	0.43	9.9	0.38	9.3	0.32	0.84	0.51 ns		
4	9.4	0.26	9.2	0.45	9.5	0.42	9.4	0.40	9.2	0.13	0.13	0.97 ns		
6	8.7	0.22	8.2	0.29	9.0	0.33	8.4	0.38	8.3	0.68	1.03	0.41 ns		
9	7.2	0.24	6.2	0.33	6.9	0.33	6.3	0.39	8.1	0.30	2.97	0.04 *	0.718	0.454
10	6.9	0.25	6.1	0.33	6.7	0.27	6.0	0.31	8.6	0.18	5.10	0.003 **	0.641	0.405
12	6.5	0.34	5.5	0.31	6.4	0.33	5.8	0.25	8.3	0.23	4.95	0.004 **	0.680	0.430
(n)	8		8		8		8		2					

Table 6.35 : Group mean haemoglobin concentrations (g/dl) over the study with ANOVA results indicating significant differences.

	Basal diet		High diet		F	P_{diet}	No work		Work		F	P_{work}	F	$P_{diet \times work}$
Week	mean	s.e.	mean	s.e.			mean	s.e.	mean	s.e.				
2	9.9	0.27	10.2	0.28	0.65	0.43 ns	10.3	0.23	9.8	0.30	1.77	0.20 ns	0.00	0.99 ns
4	9.3	0.25	9.5	0.28	0.27	0.61 ns	9.5	0.24	9.3	0.29	0.19	0.67 ns	0.00	0.95 ns
6	8.5	0.18	8.7	0.26	0.63	0.43 ns	8.9	0.20	8.3	0.23	3.11	0.09 ns	0.17	0.68 ns
8	7.1	0.24	7.2	0.22	0.13	0.72 ns	7.5	0.17	6.8	0.25	4.74	0.04 *	0.44	0.51 ns
9	6.7	0.24	6.6	0.26	0.07	0.79 ns	7.1	0.20	6.3	0.25	6.04	0.02 *	0.53	0.47 ns
12	6.0	0.26	6.1	0.21	0.01	0.90 ns	6.4	0.23	5.7	0.19	6.58	0.02 *	0.46	0.50 ns

Table 6.36 : Dietary and work effects on blood haemoglobin concentrations (g/dl) during the study with ANOVA results.

Figure 6.12 : Mean corpuscular haemoglobin levels (MCH) for each group over the experiment.

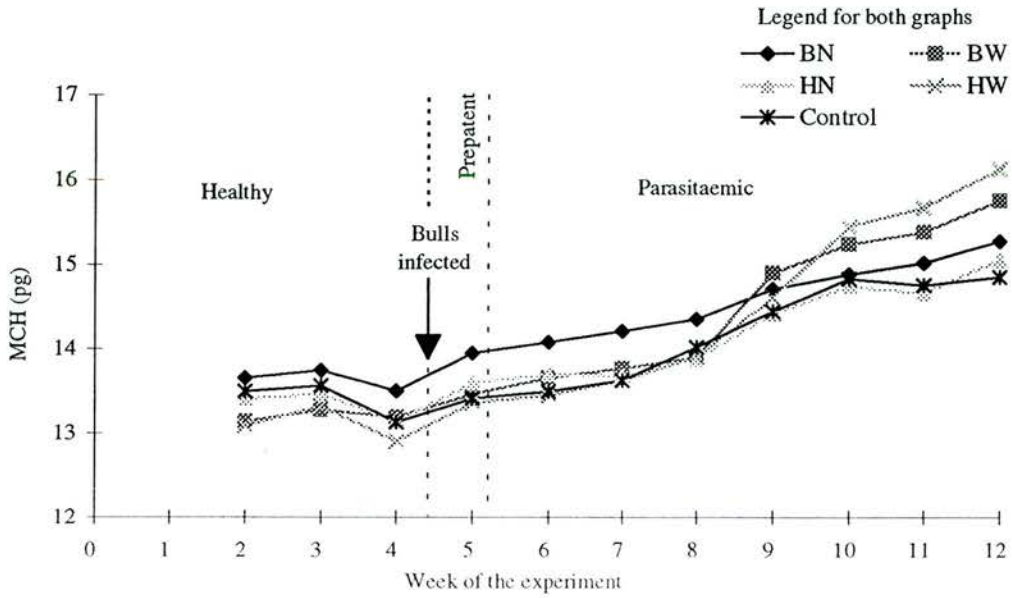
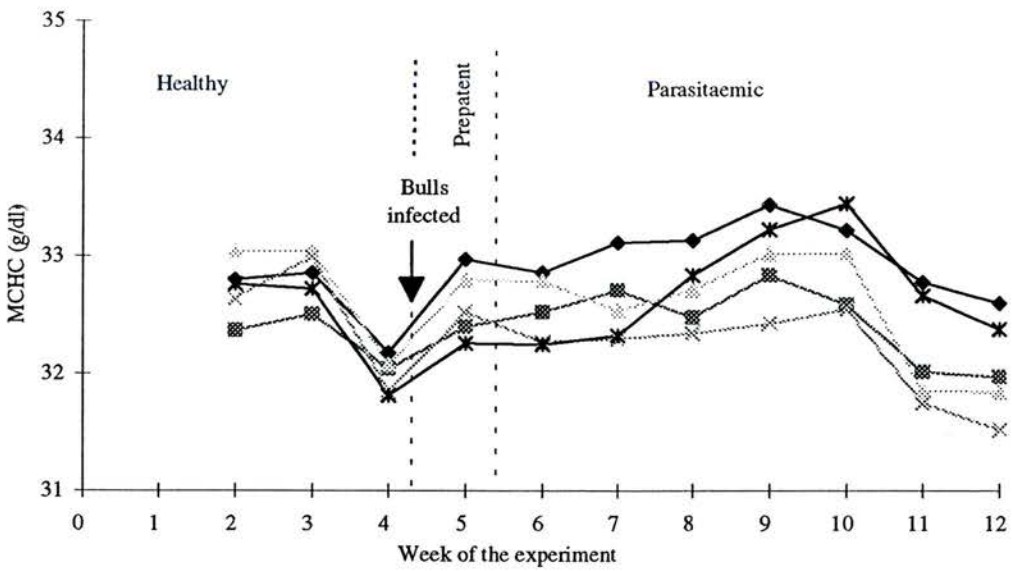


Figure 6.13 : Mean corpuscular haemoglobin concentrations (MCHC) for each group over the experiment.



6.3.10 Comparison of techniques for correcting inter-plate variation in ELISA

There were significant differences between the OD results obtained from multiple plates corrected using the F factor technique and OD results for the same sera obtained from a single plate for both the IgG_1 and the IgM assays (Wilcoxon's signed rank tests). The IgG_1 results for three of the six bulls tested on multiple plates and corrected were significantly different ($P < 0.05$) to the results for the same bulls from single plate tests, for one further animal the difference was almost significant ($P < 0.1$). For the IgM assay, the F factor correction of multiple plates produced very significantly different ($P < 0.01$) results to single plate tests for four of the six animals.

The calibration curve method of correcting for inter-plate variation also proved unsatisfactory for both assays, giving very different results to those obtained screening the sera on single plates. In some instances parasite-specific immunoglobulin levels, which were rising over time when the sera was screened on a single plate, appeared to be falling when screened on multiple plates corrected using a calibration curve and vice versa. Figure 6.14 & 7.16 illustrate the IgG_1 and the IgM results respectively, for bull 32 (HN) from single plate assays and from multiple plates corrected by both techniques. Gaps in the graphs are caused by missing data where the OD results of duplicate wells varied by more than the limits set for the assay.

Figure 6.14: Parasite-specific IgG1 response of bull 32(HN) measured by ELISA on single & multiple plates.

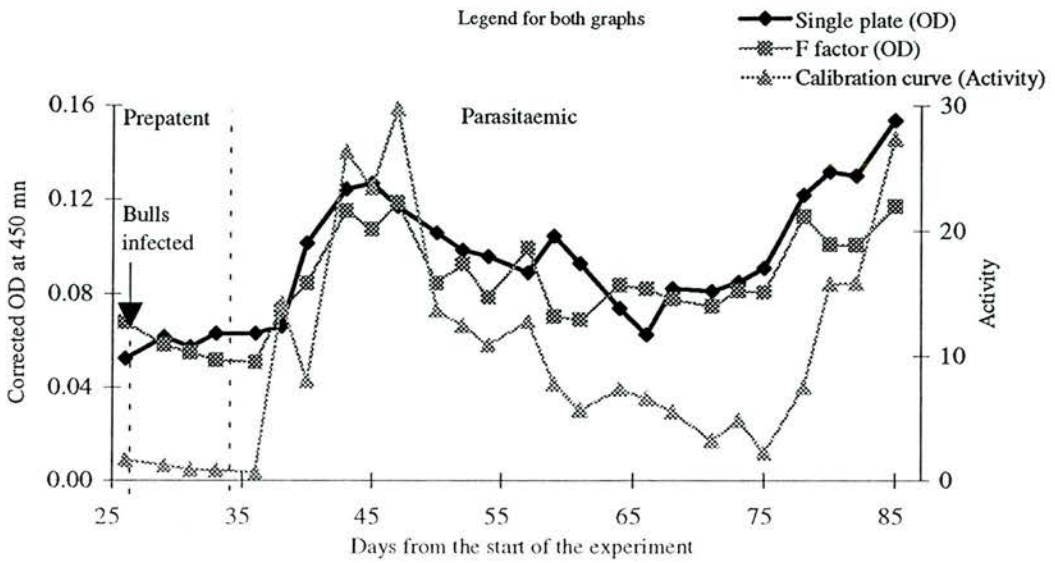
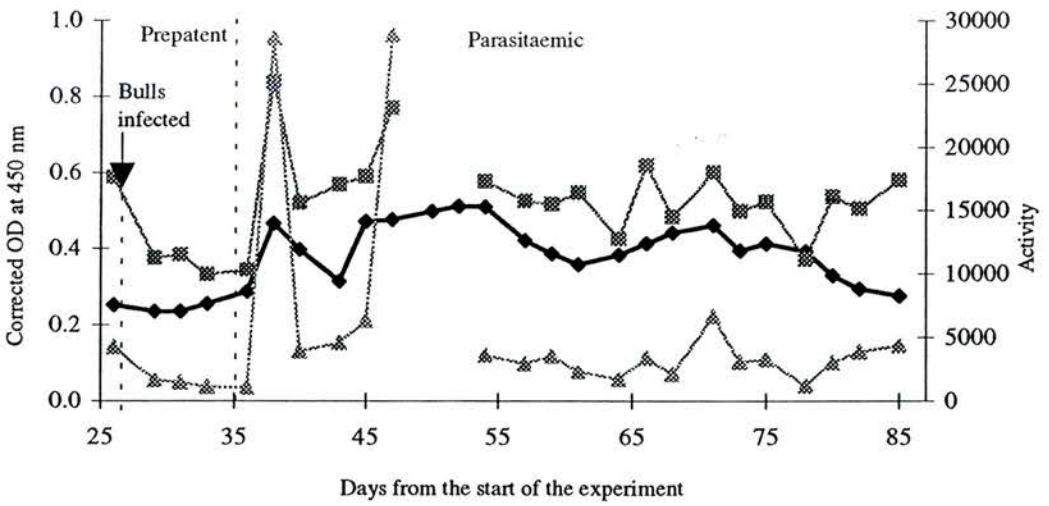


Figure 6.15: Parasite-specific IgM response of bull 32(HN) measured by ELISA on single and multiple plates.



6.3.11 Immune responses to infection

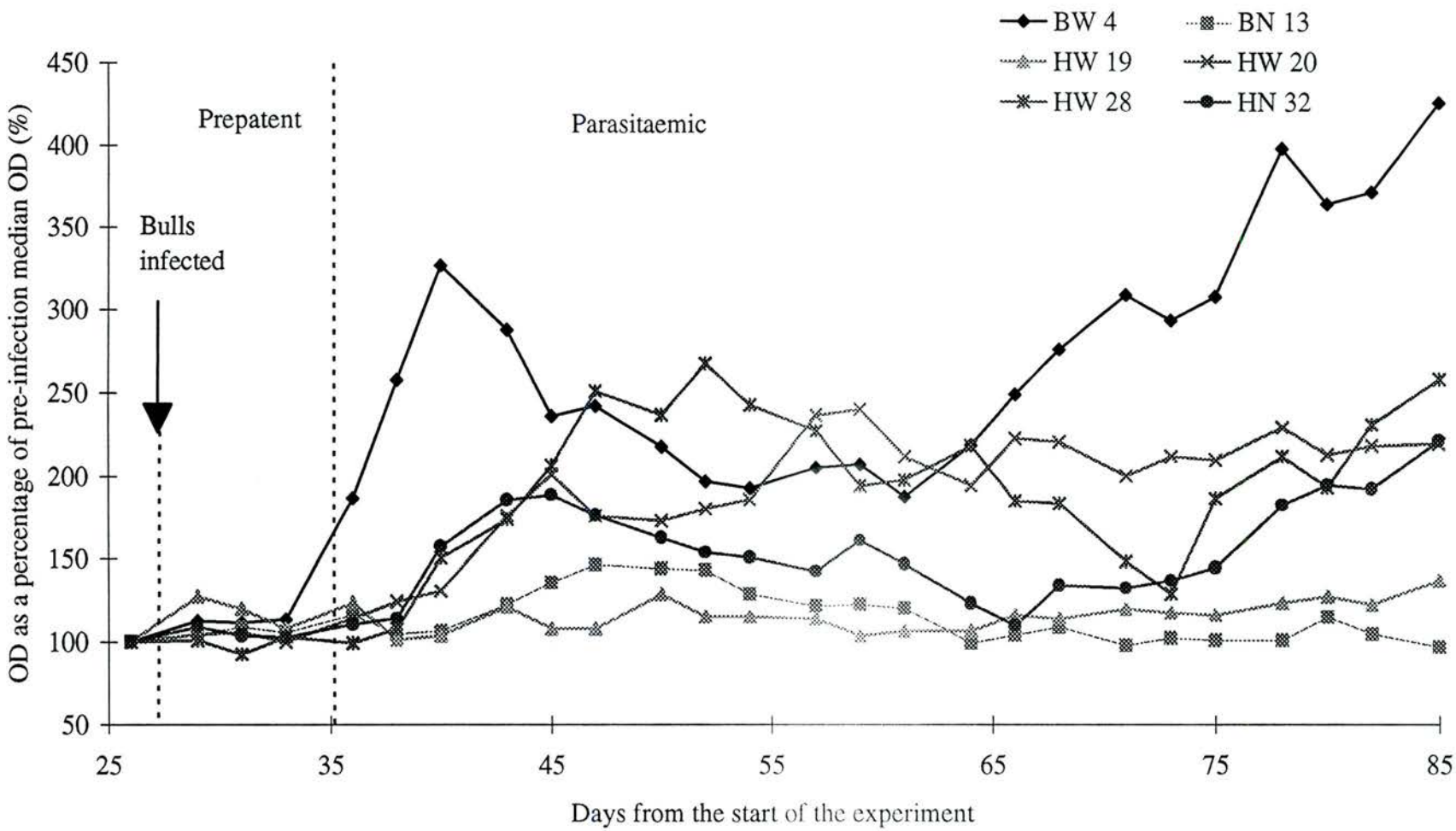
Because neither of the inter-plate correction techniques appeared to give reliable results statistical analysis of immune responses to infection was limited to ELISA OD results obtained on the same plate.

IgG_1 responses

There were no significant group differences (KW, $P > 0.05$) in *T. congolense* specific IgG_1 at any time during the experiment. Removing the data for the two bulls (HW 19 and 28) which were agglutination test positive prior to the experiment (Section 6.2.14) had no effect on the results. Group median IgG_1 titres rose slowly p.i. showing little increase until at least 20 days p.i., thereafter they continued to rise until the end of the experiment. The responses of individual bulls varied markedly regardless of group, both in the pattern and magnitude of the response. The serum IgG_1 titres of some individuals showed little or no increase p.i. those of other bulls rose sharply relative to their pre-infection medians. Some animals produced marked responses as soon as they became parasitaemic others did not appear to respond until they had been infected for 3 - 4 weeks. The IgG_1 responses of individual animals measured by ELISA are detailed in appendix 42.

The IgG_1 responses of four bulls, one from each treatment group chosen at random and each assayed on a single ELISA plate, are illustrated in Figure 6.16 together with results for bulls HW 19 and 28 (agglutination test positive). Bull BW 4 appeared to produce a 200% increase in parasite-specific IgG_1 relative to its pre-infection median within 14 days of infection and maintained a high titre for the duration of the trial. Some of the other bulls in particular BN 13 and HW 19 showed very little response to infection (the latter was agglutination positive).

Figure 6.16 : Parasite specific IgG_1 titres of six bulls p.i. measured by ELISA.



IgG_2 responses

No reliable results were obtained from this ELISA as it proved impossible to establish an assay which was sufficiently sensitive to changes in anti-*T. congolense* (ITC84) IgG_2. Despite repeatedly changing the dilutions of the assay constituents, serum samples from the experimental bulls and a variety of positive controls some of which contained enhanced levels of IgG_2 (supplied by Serotec Ltd., Oxford, UK), all produced very low optical densities (OD < 0.090), indistinguishable from the negative controls.

IgM responses

Parasite-specific IgM titres rose rapidly once the bulls were infected irrespective of group. There was an initial peak in the response at 11 days p.i. (day 38) with a higher and more persistent secondary peak between 25 and 35 days p.i. (days 52 to 62) in most bulls. Between the peaks IgM titres fluctuated although they remained elevated. By the end of the experiment the serum IgM of most of the animals had returned to pre-infection levels. The pattern of response was broadly similar in most of the bulls although the amounts of IgM produced as measured by ELISA differed considerably between animals (Appendix 43).

There were significant differences in the IgM titres of the groups on several occasions p.i., however these differences were not consistent between groups and were only transient with no significant differences seen after day 54. The pre-infection group median titres of IgM were not significantly different (KW, H = 7.3, P = 0.06), although the median of BW was 25% higher than that of any of the other groups. After infection the median IgM level of BW was significantly higher than that of HN on day 31, four days p.i. (H = 8.8, P = 0.03), higher than HW on day 54 (H = 10.61, P = 0.01) and higher than both on day 36 (H = 13.3, P = 0.004). On day 45 the median IgM level of BN was significantly higher than that of either of the high

nutrition groups (H = 12.2, P = 0.007). Removing the data from the two agglutination positive bulls had little effect on the results, although the p.i. median IgM level of BW was significantly higher than that of HW on three additional days, 52, 68 and 71 ($P < 0.05$).

Figure 6.17 illustrates the parasite-specific IgM responses from single plate tests of the same six bulls which were individually screened for IgG_1. IgM responses were more uniform than IgG_1 responses. The IgM titres of four of the six increased rapidly after infection, rising by 50% or more within 7 - 10 days. The IgM responses of bulls HW 20 and 28 were slightly slower than those of the other bulls although the size of the responses were similar. IgM titres declined slowly approximately one month p.i. (day 60).

Total IgG responses

Parasite-specific IgG titres did not differ significantly between groups at any time during the experiment, measured by the IgG (whole molecule) ELISA (KW, $P > 0.05$). Removing the data for the two agglutination positive HW bulls did not alter the significance of the results. The pattern and magnitude of responses varied markedly between bulls regardless of group. Infection caused some animals to produce large amounts of IgG whilst other produced very little. Individual responses are detailed in Appendix 44. Group median IgG titres increased steadily throughout the trial commencing as soon as the bulls became parasitaemic.

The parasite-specific responses of the six bulls whose sera were assayed on single plates are illustrated in Figure 6.18. The total IgG levels of four of the six bulls doubled within 20 days of infection (by day 47) whereas as with IgG_1 the responses of bulls BN 13 and HW 19 were less pronounced.

Parasite-specific immunoglobulin responses of individual bulls

The relative changes in class-specific immunoglobins of individual bulls are illustrated in Figure 6.19 and 7.21. The type and magnitude of the response differed markedly between individuals even within a group as shown by the responses of three HW bulls.

Figure 6.17 : Parasite specific IgM titres of six bulls p.i. measured by ELISA.

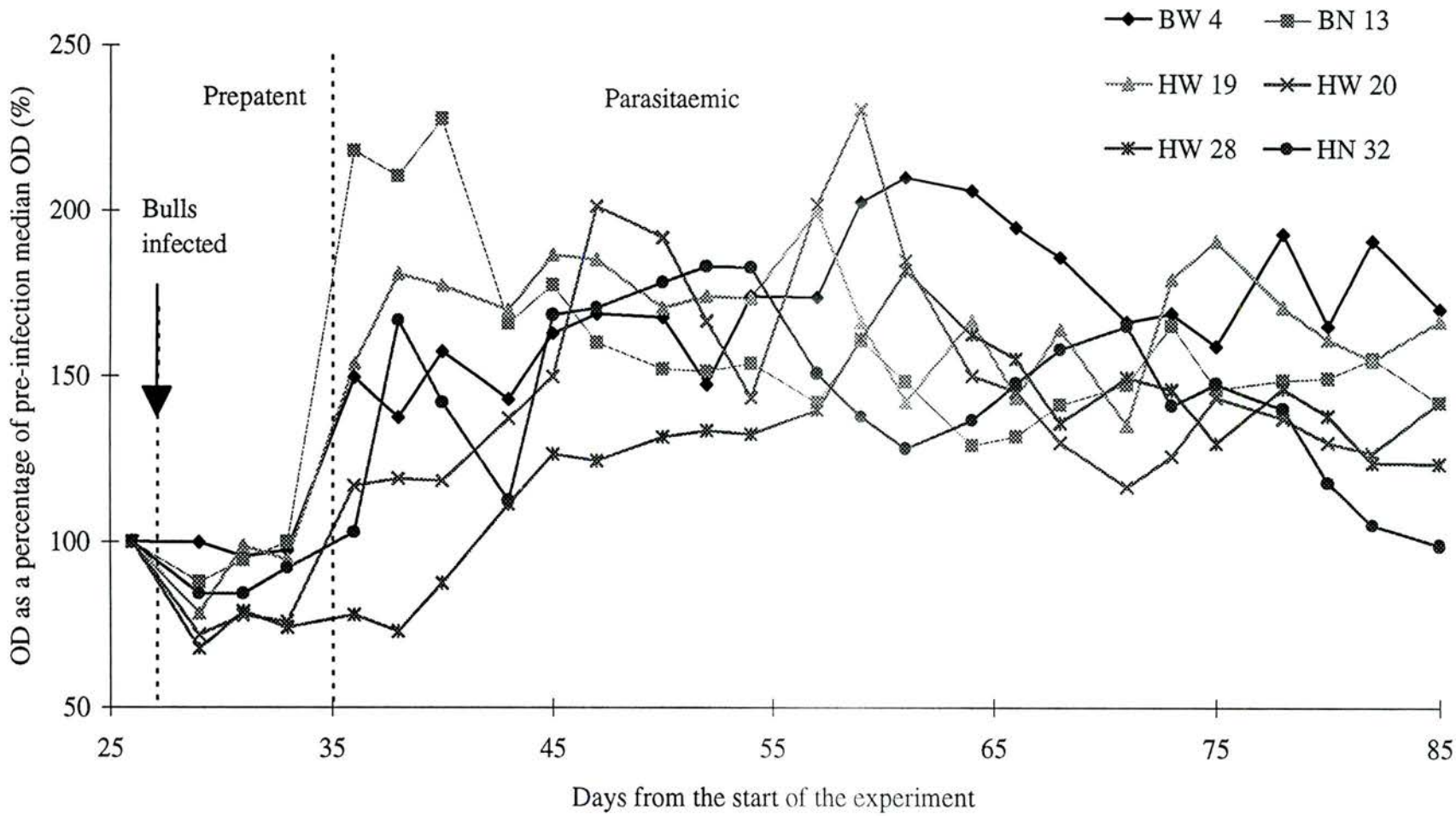


Figure 6.18 : Parasite specific Total IgG titres of six bulls p.i. measured by ELISA.

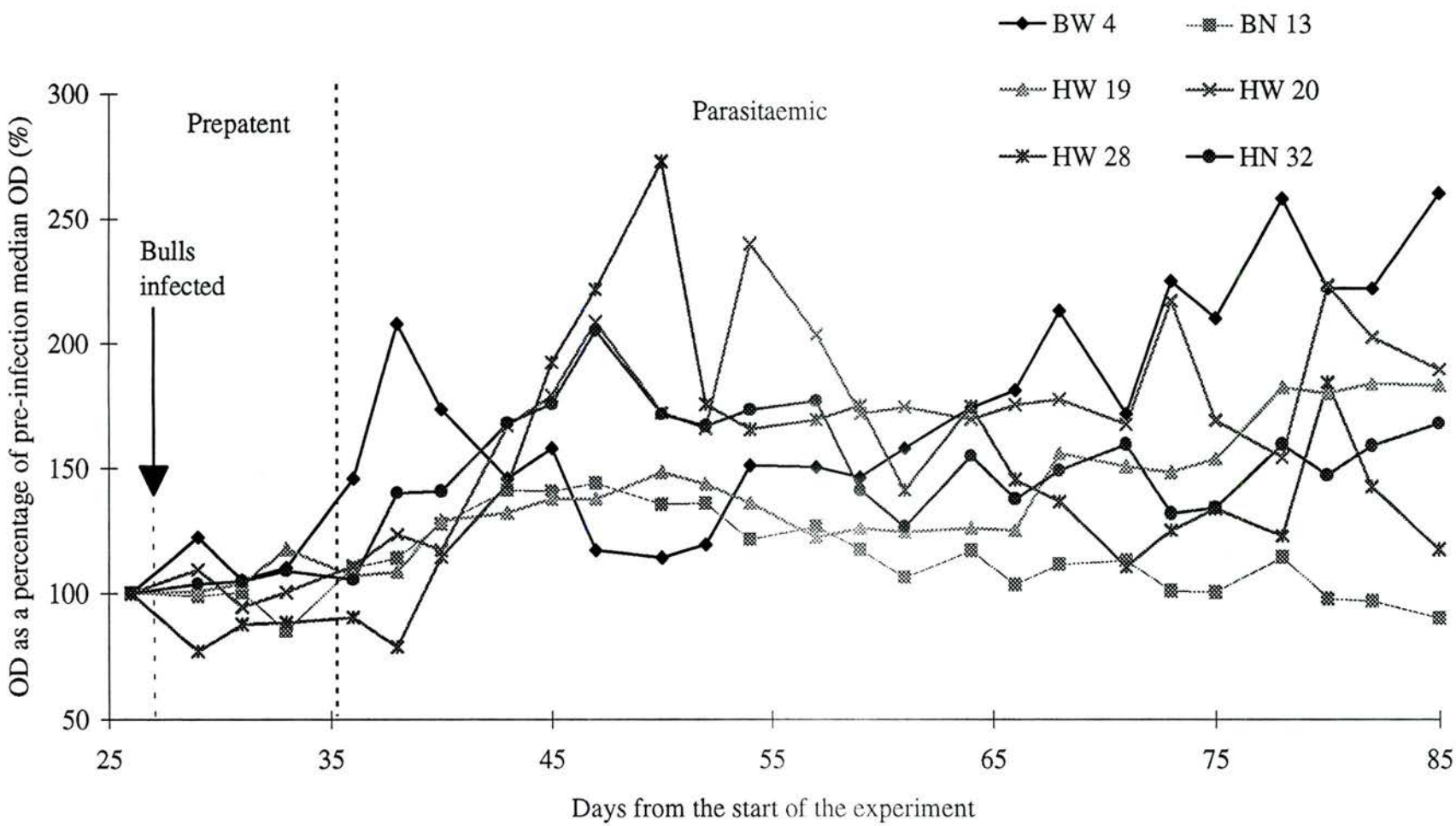


Figure 6.19: Parasite-specific immunoglobulin responses of bulls in three different groups.

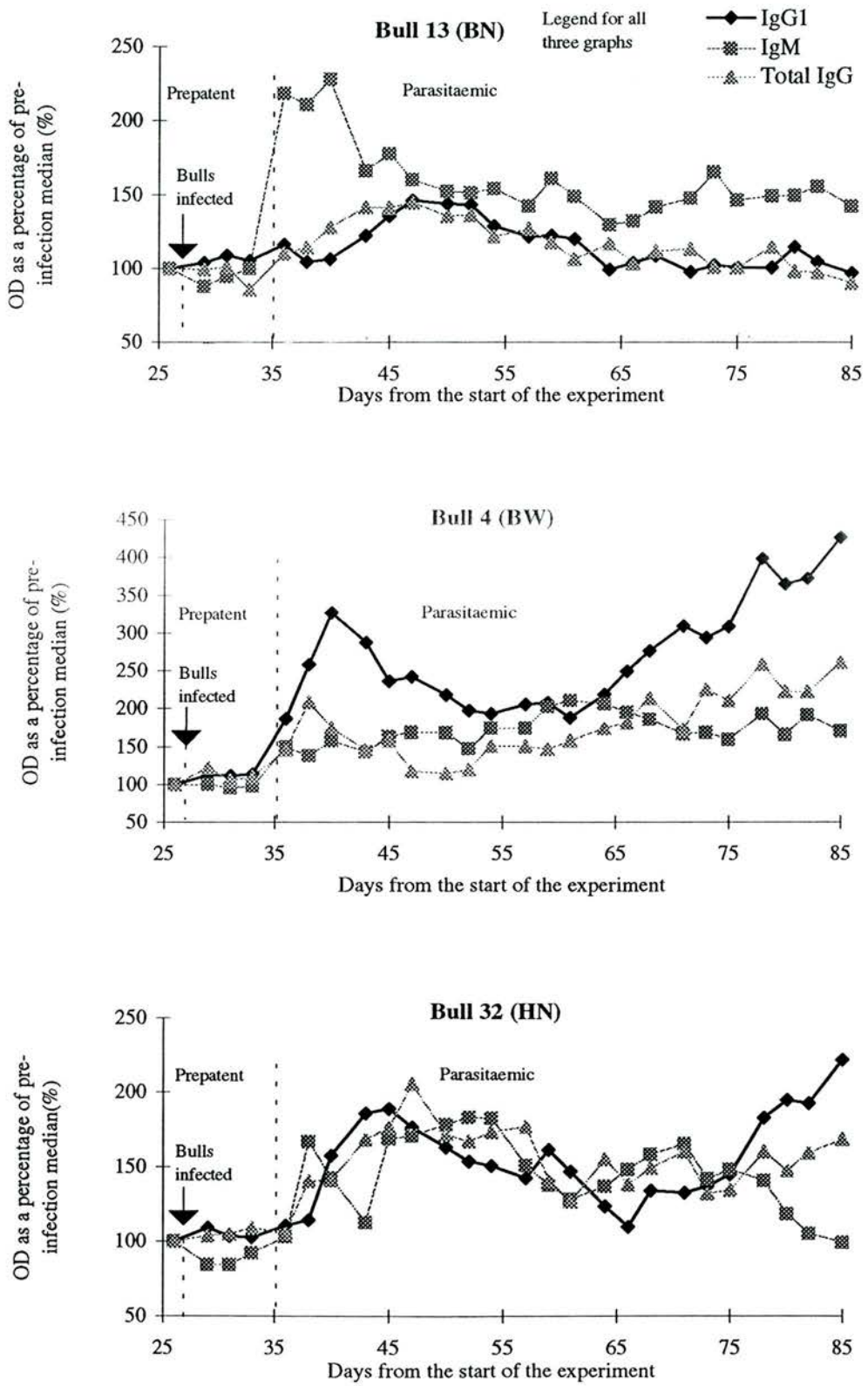
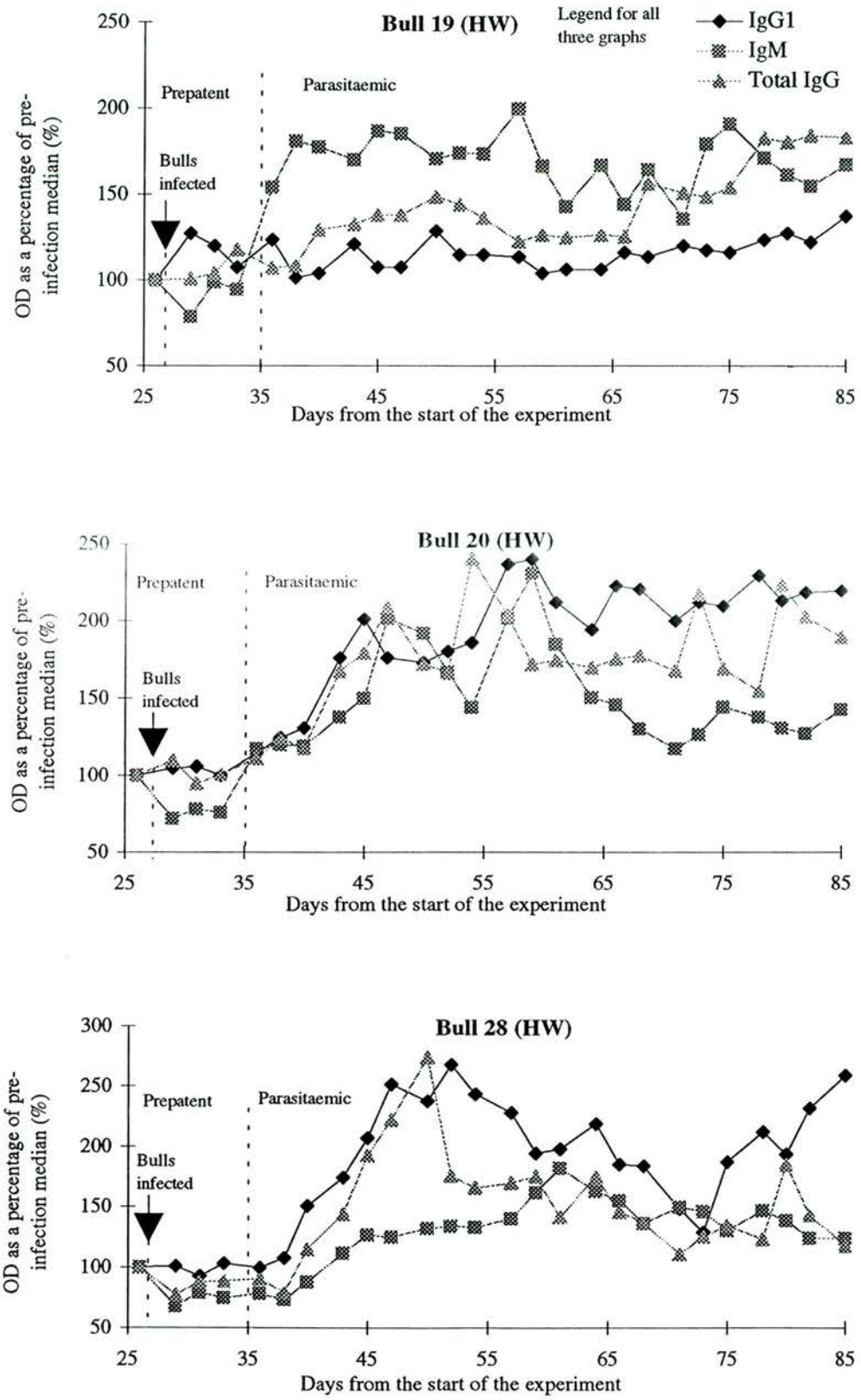


Figure 6.20 : Parasite-specific immunoglobulin responses of 3 HW bulls.



6.4 Discussion

In this study trypanosomosis caused the working speed and power output of trypanotolerant N'Dama cattle to fall by an average of 30% within four weeks of infection. Furthermore work reduced trypanotolerance, significantly increasing parasitaemia and anaemia to the point where some animals became critically ill and were unable to continue working. There were however no detectable differences in the parasite-specific antibody responses of working and non-working bulls. Infection depressed appetites causing most of the bulls to lose weight. Rates of weight loss experienced by the animals post-infection were unaffected by plane of nutrition, but were exacerbated by work.

Results from the trial confirm Gambian farmers observations that when N'Dama are infected with trypanosomes, they lose power and work more slowly (Alford, 1994). Lap speeds and power outputs fell by an average of 30% within four weeks of the bulls being infected. A decline in work performance of this magnitude at the height of the cultivation season would have serious implications for a farmer without spare draught animals. Similar reductions in performance have been reported in the few other quantitative studies of the impact of disease on the work output of draught animals. In Java Rukmana (1979) observed that buffalo artificially infected with *T. evansi* ploughed 29% less land per hour than non-infected animals. In Nepal Pearson (1989) recorded an increasing difference (up to 33%) in the power outputs of two similar teams of buffaloes carting loads over a short working season. The only apparent difference between the two teams was that the weaker pair were anaemic possibly due to a chronic sub-clinical fasciolosis, although this was not confirmed.

N'Dama infected with trypanosomes may be unable to sustain even light work for more than a few days. The ADF required by the bulls to pull their sledges during this study was not high, 6.5% of liveweight, and yet only three of the eight infected

teams were able to complete the 12 week work programme. On Gambian farms Alford (1994) found that the mean ADF generated by N'Dama cultivating fields was 10% of team liveweight (range 7 -19%) with animals walking 12 - 16 km over a 5 hour working day. In this experiment the animals walked a maximum of 8.2 km/day. N'Dama are reputed to be particularly strong in relation to body size when compared to other cattle, able to sustain ADF's up to 14% of liveweight compared to only 10 - 12% for other breeds (CEEMAT/FAO, 1972). The increasing working speed and power output of the control team over the study indicated that the work demanded of the bulls was well within the capabilities of healthy N'Dama, although it was obviously sufficient to stress the infected animals.

The working speeds of infected teams declined rapidly as parasitaemias increased, with teams working at their slowest rate 7 -10 days after the bulls' peak parasitaemias when blood PCV's were still falling. This would suggest that the trypanosomes or the immediate immune responses of the bulls themselves had a direct effect on work output. A view that appears to be strengthened by the fact that the speeds of those teams that were able to continue working, started to recover after week 8 despite the continuing decline in PCV's. Interpretation of these results is however complicated by the addition of sesame cake to all rations at the end of week 7 which provided the animals with extra energy as well as protein.

The working speeds of draught animals generally fall over the course of each day as they become tired (Pearson, 1989; Alford 1994) but the fatigue effect is not normally carried over to the following day unless animals are grossly over-worked, under-nourished, or stressed in some other way. In this experiment however once the bulls became parasitaemic they not only slowed down during each day but also over the working week, suggesting that trypanosomosis may lead to cumulative work fatigue in draught oxen. In the field such a downward spiral of reducing work output has serious consequences for the timeliness of cultivation operations particularly

where the growing season is short. Gambian farmers know that if an animal contracts trypanosomosis at the beginning of the rains it will not be able to continue working for the whole season (Alford, 1994), however they may not be aware that work performance may start to decline as soon as the animal becomes parasitaemic.

Throughout the experiment some teams worked consistently faster than others, some bulls walked faster than others and some drivers pushed their animals on where others were content to let them work at their own pace. Hence the need to use each animal as it own control when examining changes in work performance over time. Pearson *et al* (1989) reported a similar driver effect in a ploughing experiment in Nepal. Because of the small number of bulls in this trial it was not possible to determine accurately whether speed had any effect on the infected teams' abilities to complete the 12 weeks work, or on their tolerance of the infection, although observations would suggest it had little effect on either parameter. Teams 1 and 4 were fast teams, teams 2 and 3 slow teams, 1 and 2 retired after 9 and 8 weeks work respectively, 3 and 4 managed 11 and 12 weeks.

The energy expenditures of the working bulls in the study 1.37 - 1.41 $\times$ ME_{maint} were similar to figures recorded elsewhere (Lawrence, 1985; Pearson *et al*, 1989). Lawrence (1985) suggested that $1.7 \times ME_{maint}$ was an optimal level of expenditure for working oxen. N'Dama on Gambian farms appear to be worked relatively hard; Alford (1994) found oxen used 1.8 - 2.0 $\times$ ME_{maint} when employed on cultivation tasks. Working cattle at such high levels of energy expenditure might have an adverse effect on their tolerance should they become infected with trypanosomes, necessitating their immediate withdrawal from work. The danger of overwork causing a breakdown in the trypanotolerance of N'Dama was recognised by Starkey (1982) who stated that high levels of work require particularly careful management of the animals.

Trypanosomosis appeared to cause a significant increase in the bulls' body temperatures in this study which may have been a contributory factor reducing the work output of the working animals. Animals starting work with elevated body temperatures will become heat stressed more rapidly than those starting work with normal temperatures, although this may be less of a problem in N'Dama cattle than in other breeds, since N'Dama are reputed to be particularly heat tolerant (Murray *et al*, 1979). There is conflicting evidence as to whether or not trypanosomosis actually causes fever in N'Dama cattle. Murray *et al* (1981) stated that N'Dama naturally infected with trypanosomes seldom become febrile, however the majority of the cattle in their study were infected with species other than *T. congolense*. Other authors have recorded elevated rectal temperatures in N'Dama infected with a variety trypanosome species including *T. congolense* (Ige and Amodu, 1975; Dwinger *et al*, 1992). Greig and McIntyre (1979) suggested that the routine recording of rectal temperatures is unrewarding because of the large diurnal fluctuations that can occur in healthy N'Dama, from as low as 34.4°C at dawn to 41.1°C in late afternoon. In this trial prior to infection temperatures ranged from 34.2 to 41.3 °C, the maximum temperature recorded p.i. was 41.6°C, that was after work.

Working bulls had significantly higher parasitaemias and subsequently became more anaemic than non-working animals, with significantly lower PCV's from day 35 p.i.. Studies by Dargie, Murray, Murray, Grimshaw and McIntyre (1979) have shown that PCV is a good measure of trypanosomosis induced anaemia. The trypanotolerance of N'Dama cattle has been characterised as a superior ability to control parasitaemia and resist anaemia when infected with trypanosomes (Dargie *et al*, 1979; Trail, d'Ieteren and Teale, 1989; Paling, Moloo, Scott, Gettinby, McOdimba and Murray, 1991). Hence the conclusion that work appears to reduce the trypanotolerance of N'Dama cattle.

The severity of the anaemia observed in the infected, working animals compared to their non-working contemporaries may have been caused at least in part by their higher body temperatures after work. Exposing red blood cells to temperatures only a few degrees above normal for only a few hours increases the permeability and osmotic fragility of the cells, reducing their plasticity, making them easier to destroy (Karle, 1974). This led Murray and Dexter (1988) to suggest that febrile responses to trypanosome infections might increase the rate of red cell destruction. In this study the combined effects of fever and work induced increases in body temperature may have decreased erythrocyte half-life still further.

The observation that plane of nutrition had no significant effect on parasitaemia nor on PCV, appeared to contradict findings from other experiments which indicated that the severity of trypanosome infections could be reduced by improved nutrition, however closer examination showed that the results were not directly comparable. Little *et al* (1990) found that a high plane of nutrition reduced the rate of decline of PCV immediately p.i. in N'Dama infected with *T. congolense*, particularly in animals in poorer condition, although it had no effect on parasitaemia. The crude protein concentrations of the high and the low diets fed by Little *et al* (1990) were however more than double the mean p.i. crude protein concentrations of the high and basal diets in this experiment, even after the addition of sesame cake. The ME intakes of the animals in this study were intermediate to the two levels fed by Little *et al.* In another study Agyemang *et al* (1990b) observed that N'Dama grazing natural pastures recovered more rapidly from anaemia induced by tsetse-transmitted trypanosome infections when they were given a concentrate supplement, but the difference in rate of recovery was not significant until 16 weeks p.i.. This study finished 8 weeks after infection. Unfortunately neither Little *et al* (1990) nor Agyemang *et al* (1990b) gave details of feed intakes nor previous trypanosome infection histories of the cattle they used. Katunguka-Rwakishaya, Parkins, Fishwick,

Murray and Holmes (1993) reported that high levels of protein in isoenergetic diets led to increased parasitaemias in Scottish Blackface sheep infected with *T. congolense,* but had no effect on the degree of anaemia and actually enhanced the rate of recovery following chemotherapy. In summary, the responses observed in all three of the studies cited above were probably directly attributable to increased protein intakes rather than improved nutrition *per se*. Blood PCV's are known to be strongly correlated to protein intake (Pelletier, Tremblay and Hélie, 1985), erythropoiesis is markedly reduced by low protein intakes (Reissman, 1964). In this experiment rations were formulated to ensure two very different ME intakes, with similar crude protein intakes. Future studies on the interactions of nutrition, work and trypanotolerance should utilise isoenergetic diets with varying levels of protein.

The strong positive correlation observed between PCV's at the start and end of the experiment amongst the infected bulls, implies that in areas where trypanosomosis is endemic farmers with more than two draught N'Dama should work those with the highest PCV's at the start of the season, to reduce the risk to the animals should they become infected. Without access to laboratory facilities however such advice is of little use. A more practical suggestion is that farmers with only two animals might consider feeding a high protein supplement immediately before the work season to try to boost PCV's. A PCV of 15% is considered the threshold below which N'Dama are likely to die (Murray and Dexter, 1988; Authié *et al*, 1993b). PCV's of trypanotolerant livestock under tsetse challenge have been found to be positively correlated to production traits such as reproduction and growth (ILCA, 1986). Using PCV as a selection criterion for trypanotolerance as suggested by Murray and Dexter (1988), Paling *et al* (1988) and Dwinger *et al* (1992) would not only benefit these traits but should also improve draught performance; infected animals could be worked for longer before they became seriously anaemic.

The variations in parasitaemias and PCV's observed once the bulls became parasitaemic were reflections of initial PCV's coupled with differences in the degree of trypanotolerance which in some individuals may have been enhanced by acquired immunity (Desowitz, 1959). Similar variations have been observed in other studies (Murray *et al* 1981; Paling *et al*, 1988; Dwinger *et al*, 1992). As early as 1977 Murray *et al* (1977b) and Clifford and McIntyre (1977) concluded that a wide range of susceptibilities to trypanosomosis exist within the N'Dama breed. The cattle used in this experiment were selected as typical of the N'Dama breed in The Gambia. Nothing was known of their genetic background nor previous infection history with respect to trypanosomosis or any other disease. The original intention had been to screen sufficient bulls to obtain at least 32 animals of similar immunological status, namely low *T. congolense* antibody titres and no VAT antibodies to the infecting stabilate (ITC84), however, a shortage of suitable bulls coupled with financial constraints meant that only 40 bulls could be purchased for screening. Unfortunately there were insufficient similar animals in the ITC station herd to replace all those that proved antibody positive.

The correlation between parasitaemia (quantified as mean parasitaemia score) and anaemia (mean PCV once infected) seen in this study confirmed Murray and Dexter's observation (1988) that parasitaemia affects the severity of the subsequent anaemia. A number of experiments have however clearly demonstrated that the ability of trypanotolerant livestock to resist anaemia is not directly linked to a superior ability to control parasitaemia, but that the two are independent traits (Wellde, Hockmeyer, Kovatch, Bhogal and Diggs, 1981; Murray *et al*, 1982; Murray and Dexter, 1988; Paling *et al*, 1988; Paling *et al*, 1991a).

Although trypanotolerance was reduced by work, it did not breakdown completely in any animal. All the bulls that ceased work early were maintained on their experimental diets, none required early treatment with trypanocide and none

died. Within 35 - 40 days of infection the PCV's of most of the 32 animals had stopped falling, a pattern observed in other trials for example by Paling *et al* (1988). Unfortunately this particular study was not continued for long enough to determine whether any of the bulls would self-cure with PCV's returning to normal, as reported elsewhere in the literature (Murray *et al*, 1977b; Murray *et al*, 1979).

The WBC counts of the infected animals p.i. followed the classic pattern seen in cattle with *T. congolense* and *T. vivax* infections, namely a rapid leukopaenia between infection and the first parasitaemic peak, followed by a leukocytosis approximately 3 - 4 weeks p.i. (Naylor, 1971; Losos, Paris, Wilson and Dar, 1973; Valli and Mills, 1980; Saror *et al*, 1981; Ellis, Scott, Machugh, Gettinby and Davis, 1987). The leukopaenia is caused primarily by a decrease in lymphocytes and neutrophils (Naylor, 1971; Saror *et al*, 1981; Ellis *et al*, 1987).

The slight decline in the mean PCV, RBC count and haemoglobin concentration of the uninfected control group over the course of the study may have been caused by work. Long-term training and endurance exercise are known to cause PCV's and haemoglobin concentrations to fall slightly in humans (Kaiser, Janssen and van Wersch 1989) leading to a condition known as sports anaemia (Yoshimura, 1970). The reason for the slight decline in the WBC counts of the two control bulls over the course of the study is less clear. Short periods (lasting no more than a day) of acute exercise have been associated with transient leukocytosis in cattle (Singh *et al*, 1968; Pearson and Archibald, 1989), however there appears to be a scarcity of information on the effects of prolonged exercise. Heat stress *per se* has also been reported to increase blood leukocyte numbers in mature cattle (Wegner, Schuh, Nelson and Stott, 1976) although it had no effect in calves (Kelley *et al*, 1981).

The bulls' parasite specific serum IgM titres rose rapidly as soon as the animals became parasitaemic, whereas IgG_1 titres increased more slowly commencing at least 20 days p.i.. This is a typical response pattern in cattle infected with

trypanosomes, the interval between infection and an increase in IgG_1 varying between 20 and 60 days depending upon the duration of prepatency (Clarkson and Penhale, 1973; Authié *et al*, 1993b).

The reduced trypanotolerance observed in the working bulls compared to the non-working animals would suggest that both variant specific anti-VSG (variable surface glycoprotein) and invariant antibody production may have been depressed by work. Variant specific anti-VSG antibodies are believed to control parasitaemia (Roelants and Pinder, 1984) whilst invariant antibody responses are thought to be important in resisting the pathogenic effects of the disease (Authié *et al*, 1993b). The absence of significant differences in group immunoglobulin responses might be explained by the considerable individual variation observed within groups masking group differences. The magnitude of immunoglobulin responses, particularly IgM responses, can vary considerably between individuals (Clarkson *et al*, 1975). Recently however, Authié (1994) proposed that parasitaemia may be controlled by a number of immunological and non-immunological factors, not just anti-VSG antibodies, after observations that there was no difference in either the isotypes or titres of anti-VSG antibodies produced by trypanotolerant and susceptible animals challenged with *T. congolense* (Authié *et al*, 1993b). Authié's hypothesis may offer a better explanation for the results obtained in this study.

The total IgG responses of the bulls p.i. appeared relatively small when compared to IgG_1 and IgM responses. This agrees with the observation of Authié *et al*, (1993b) namely that although total serum IgG can increase in trypanosome-infected cattle as demonstrated by Clarkson and Penhale (1973), increases tend to be moderate and are not a consistent feature of such infections.

The failure of the ELISA used to monitor the bulls' IgG_2 responses was not of major concern given the findings of Authié *et al*, (1993b) and Kobayashi and Tizard, (1976) suggesting that IgG_2 responses to trypanosomal challenge are minimal. There

was no obvious reason why this assay did not work, although a very small IgG_2 response coupled with competitive inhibition by the much larger quantities of IgG_1 in the sera (Kemeny, 1992) may have contributed to the problem. The anti-bovine IgG_2 monoclonal antibody used in the ELISA was checked and shown to be working satisfactorily using a Dotblot assay (appendix 45).

In this study work did not have a consistent, significant effect on parasite specific immunoglobulin titres. In the literature there are conflicting reports on the effects of exercise on immunoglobulin titres, complicated by such factors as level of fitness and the nature of the exercise, maximal or sub-maximal, prolonged or short-lived. Wong *et al*, (1992) found that a single bout of intense exercise had no effect on serum IgG, IgM and IgA levels in unconditioned horses, whereas chronic exercise at a lower intensity has been demonstrated to increase pathogen specific immunoglobulins in mice (Liu and Wang, 1987). In humans however, both increases and decreases in immunoglobulins have been reported post-exercise (Poortmans, 1970; Poortmans and Haralambie, 1979; Tomasi *et al*, 1982; Tharp and Barnes, 1990; Nehlsen-Cannarella *et al*, 1991; McDowell *et al*, 1992). Unfortunately there appear to be no reports on the effects of long-term exercise on immunoglobulin responses in ruminants.

Plane of nutrition did not affect parasite specific immunoglobulin responses possibly because the degree of undernutrition imposed on the animals in the low nutrition groups was not severe enough. Fiske and Adams (1985) reported reduced antibody response to ovalbumin in steers losing 500 g /day when compared to control animals fed for a slight weight gain. In this experiment the mean weight loss of BW, the most nutritionally stressed group, during period 3 when the animals were parasitaemic was only 270 g/d.

Despite careful optimisation of each ELISA by chequerboard titration neither of the techniques used to correct inter-plate variations proved satisfactory, consequently both were abandoned. This failure may have been due to the instability

of the assays or alternatively to limitations in the correction techniques applied. Herbert, Edwards, Bushnell, Jones and Perry (1985) stated that even with a carefully set up ELISA where all constituent dilutions have been optimised by chequerboard titration, there are often significant variations in the OD's of control samples, plate to plate and particularly day to day. In this study eight hundred p.i. serum samples were collected, requiring a minimum of 25 plates to be run per assay assuming no repeats. In the three ELISA's successfully completed the coefficients of variation of the positive and negative control samples between plates were relatively large, up to 39%, (appendices 5, 6 and 7) despite the quality control measures adopted. Typical coefficients for ELISA control samples are between 10 and 20% (Kemeny, 1992). There are number of possible explanations for the variations observed in this study. Kemeny (1992) suggested that poor or uneven coating of plates is the most common source of variation and problems in ELISA's. Competitive inhibition caused by the presence of other classes and sub-classes can also increase the variability of results obtained when testing sera for specific immunoglobulins (de Savigny and Voller, 1980). This is a particular problem with IgM assays, since IgG antibodies have a much higher affinity than IgM and consequently any IgG present in the test serum may disproportionately inhibit binding of IgM to antigen (Kemeny, 1992). Competitive inhibition may have led to the low binding ratios seen in this IgM assay, resulting in a loss of accuracy (Voller *et al*, 1979). Physical separation of immunoglobulins by filtration chromatography can solve the problem of competitive inhibition, however the techniques used have limitations and are not practical for large numbers of samples (Chantler and Diment, 1981). Non-specific binding of proteins which reduces assay sensitivity (Kemeny, 1991) was not a problem in any of the ELISA's performed in this study; negative control values were similar to background values obtained from wells containing only washing buffer.

In the absence of differences in humoral responses, future studies on immune responses to trypanosome challenge in working cattle should focus on cellular responses. Authié (1994) investigating the mechanisms responsible for trypanotolerance suggested that the cysteine protease congopain warranted further investigation along with the immunoglobulin switch which occurs part way through an infection triggered by cytokines released from helper T-cells. Cytokines are known to be adversely affected by glucocorticoids (Hoffman-Goetz, 1992) which are released as part of the second stage of an animal's response to a stressor (Section 2.2.4). Thus work stress may reduce trypanotolerance through its action on cytokine responses.

Trypanosomosis had a profound effect on the ability of the working animals to meet their energy requirements for maintenance and work irrespective of diet. Excluding weight changes over the first week of the experiment when the bulls were probably not fully acclimatised to the experimental conditions, prior to infection the mean weight gain of the bulls on the high plane of nutrition was three and a half times that of those on the low plane; a difference which was almost significant. In contrast work did not affect liveweight change pre-infection as the working animals were able to consume sufficient andropogon to meet their energy demands for work and growth. Once the bulls became parasitaemic, plane of nutrition had no effect on weight loss but work exacerbated losses. This has serious implications for farmers' animals. It suggests that if N'Dama oxen contract trypanosomosis during the working season they may lose weight regardless of what they are fed, and that the only way to reduce or halt that loss is to stop working them until appetites recover. Pronounced weight losses will ultimately affect work output even if animals are able to continue working, because the draught force an animal is capable of generating is related to its liveweight as noted in section 2.1.2.

Improved feeding or supplementation with better quality forages is unlikely to be effective in stopping weight loss in working N'Dama with trypanosomosis because

of the severe loss of appetite which is symptomatic of such infections. In this study rations were formulated to ensure that bulls on the high plane of nutrition were offered sufficient groundnut hay and rice bran to meet their estimated ME demands for maintenance and work, regardless of andropogon grass intake, whereas animals on the low plane had to eat substantial quantities of andropogon grass to meet their energy demands. Despite this, once they became parasitaemic both working and non-working animals on the high plane of nutrition lost weight. The marked loss of appetite seen in the infected groups p.i. and in particular the fall in andropogon intake was similar to that observed by Romney, N'Jie, Clifford, Holmes, Richard and Gill (1993) in a nutrition trial with N'Dama heifers artificially infected with another *T. congolense* clone.

In the present study bull liveweight losses after infection were more pronounced than might have been predicted from feed intake data. HN, the group under least nutritional stress lost an average of 250 g LW/day over the first three weeks p.i. despite an estimated mean ME intake of 1.35 × ME_{maint} (34.9 MJ/head/day). The discrepancy may have been caused by using figures for European taurine breeds (Agricultural Research Council, 1980; AFRC, 1993) in the energetics calculations, although this appears unlikely. It is more likely however that the trypanosome infections reduced the efficiency of nutrient utilisation as previously observed by Little *et al* (1990) or increased energy requirements for maintenance (Verstegen Zwart, van der Hel, Brouwer and Wensing, 1991). Bennison (1994) suggested that the animal liveweight losses during the experiment may have been greater than predicted because of a shortage of protein in the rations inhibiting rumen function. This discrepancy between estimated ME intakes and liveweight changes observed may actually have been even larger than calculated as N'Dama are reputed to be better at utilising food than other breeds of cattle and require less energy for maintenance, although neither of these traits appears to have been quantified (Murray, 1988).

The ME_{maint} requirements of the working bulls were assumed to be the same as those of the non-working animals, however recent studies have suggested that draught animals may require more energy for maintenance, particularly at low feeding levels (Lawrence, Buck and Campbell, 1989; Lawrence, Sosa and Campbell, 1989; Pearson and Dijkman, 1994). Furthermore it has been suggested that work may reduce the efficiency of utilisation of absorbed nutrients in draught ruminants by increasing the demand for scarce glucogenic precursors (Lawrence and Becker, 1995). These factors coupled with the possible effects of trypanosomes on maintenance energy requirements and the efficiency of utilisation of nutrients, may explain the large weight losses seen in the working bulls. Unless farmers have access to high quality energy dense feedstuffs it may be difficult for them to meet the energy requirements of working animals which become infected with trypanosomosis even if the animals' appetites do not decline. Further nutrition studies of N'Dama with and without trypanosomosis are needed to accurately determine the energy requirements of such animals for maintenance, work and growth.

The rates of weight loss of some animals post-infection were so severe that they were not thought to be sustainable for the duration of the trial; once a working bull lost 15% of its initial liveweight it was to cease work on welfare grounds. Animals in BW lost an average of 4% of liveweight (8 kg) over the first 21 days p.i.. Sesame cake was introduced on day 48 to provide additional protein. This proved successful in reducing weight losses, however the weekly weight losses of most animals remained higher than predicted from intake data, strengthening the suggestion that the trypanosome infections affected energy metabolism. In contrast the uninfected controls gained a mean of 13 kg liveweight over the 12 weeks of the experiment confirming that the high plane of nutrition was more than adequate for the work asked of the bulls assuming a normal appetite and normal utilisation of nutrients consumed.

The weight losses of the infected animals were large compared to those seen in other studies of *T. congolense* infections in N'Dama (Little *et al*, 1990; Romney *et al*, 1993). This may have been due to differences in the pathogenicity of the trypanosome serodemes used in the various studies. The mean weight losses of the working bulls were however similar to losses experienced by Gambian farmers' animals during the working season. Alford (1994) recorded a mean loss of 22 kg over a two month working season (0.36 kg/d), in a survey of 63 N'Dama oxen on Gambian farms. Unfortunately he did not record the health status of these animals, although in view of the area in which the studies were conducted it is likely that some of them were harbouring trypanosome infections.

The transient weight losses experienced by the bulls over the first week of the trial were attributed to the stress of commencing the experiment. Losses were highest amongst the working animals implying they had not been sufficiently acclimatised to the work beforehand. Although the bulls had been trained for one month before the study, they did not work five days a week and seldom completed the full four laps of the track during that time. It is difficult to determine the optimum acclimatisation period for draught animal studies, too long and the animals may become fatigued before the study starts, too short and the animals may not be fully fit and accustomed to the work demanded of them.

The experimental design and number of bulls in each group in this study was a compromise between the minimum number of individuals needed to achieve statistically valid results and the maximum number of teams that could feasibly be worked simultaneously but independently of each other under the same field conditions. This is a common problem in draught animal experiments, recognised by Lawrence and Pearson (1993). The control team was included primarily to monitor the work required to pull the sledges ensuring that it remained constant for the duration of the experiment, rather than for comparison with infected bulls. An

infected team could not used to monitor work in case it was unable to work for the full 12 weeks.

In view of the considerable between animal variation in a number of the parameters measured in this experiment, particularly immunological and haematological responses, future studies should use larger groups of animals or alternatively animals with a more uniform genetic background and immunological history. Here there was insufficient knowledge of the bulls' previous infection history and breeding to determine whether some responses were due to factors imposed in the experiment, innate differences in trypanotolerance or previous exposure to trypanosomes. Working larger groups of cattle obviously increases the logistical problems if meaningful data on work output is to be collected.

In conclusion this experiment has shown that there is a strong antagonistic effect between work and disease in trypanotolerant N'Dama cattle which results in a marked decline in work performance and a fall in trypanotolerance. The mode of interaction requires further investigation. A first step would be to examine the effects of work and / or trypanosomosis on cellular immunity. The study also demonstrated that working N'Dama whilst they are infected with trypanosomes is likely to lead to a loss of body weight and condition which may be impossible to stem unless the animals cease working. The results suggest that in tsetse fly infested areas of sub-Saharan Africa where rainfall is low and the planting season is short, draught cattle should be given prophylactic protection against trypanosomosis prior to the working season even if they are normally regarded as trypanotolerant. Failure to do so could seriously reduce crop yields.

7. CONCLUSIONS & RECOMMENDATIONS

To date there have been very few quantitative studies on the interactions of work, nutrition and disease in draught animals. This is probably due to the difficulties involved in quantifying such interactions because of the large number of variables which can affect each parameter. Many of these variables were apparent during the course of the three studies reported in this thesis, some were controlled, others were monitored. Despite these difficulties it is possible however to draw some conclusions from the work done, to make some suggestions for further research and perhaps more importantly to make some preliminary recommendations for farmers and others working draught animals in the field.

T. evansi in working buffalo

This study did not prove conclusively that *T. evansi* infection affects the work output of swamp buffalo. There was a pronounced decline in the work output of the buffalo towards the end of the study, however it was observed with both infected and uninfected animals. The work output of all six teams fell by 40% in only ten days, commencing 45 days after group 2 animals were infected. This was after the infected animals had worked normally for four weeks showing no overt signs of disease. It is possible particularly in view of the short track used for the study, that this general decline in output was due to a peer effect initiated by a fall in the output of one or more of the infected teams triggered by rising parasitaemias in some of the animals, although this remains only speculation. Alternatively the decline in work output observed may be attributable to fatigue or some other factor which affected all animals equally irrespective of infection status. Further studies, using a longer track

which would allow animals or teams to be worked totally independently of each other, are required to prove whether or not surra affects work output.

If trypanosomosis does depress work output, a fall of the magnitude observed here, occurring in the middle of the cultivation season could have very serious consequences for farmers without spare animals, particularly if the growing season is short. The absence of obvious signs of ill-health before output fell suggests that farmers are unlikely to realise that their animals are sick until it is too late. This may be true of a number of chronic, sub-clinical infections which can afflict draught animals. If surra is proven to reduce work output, further studies will be required to investigate whether treatment with trypanocide once output starts to decline will reverse that decline and if so how rapidly? Should chemotherapy not lead to a rapid improvement in output, farmers might be well advised to consider prophylactic protection for their draught animals before critical work periods.

The decline in the work output of the group 2 buffalo coincided with marked increases in the parasitaemias of three animals, one from each team. Unfortunately it was not possible to determine whether this decline was triggered by cumulative work fatigue depressing buffalo immune responses, which in turn allowed parasitaemias to rise or whether the increases in parasitaemia approximately 45 days p.i. were characteristic of infection with this particular *T. evansi* isolate. If the reduction in work output observed was caused by cumulative fatigue affecting immune responses, farmers who only work their buffalo sporadically or for very short periods with light loads might be able to use animals infected with trypanosomes without incurring a loss of output; the infected animals in the study worked for 4 weeks post-infection before outputs declined. Adopting such a strategy obviously carries a high risk, since work output may decline rapidly with little warning and furthermore work stress may affect the subsequent pathogenesis of surra. The course of the disease once work stopped was not considered as part of this study, although it should be noted that all

animals made a full recovery after treatment with a trypanocide once work ended. In any future study a group of unworked buffalo should be infected with the same *T.evansi* isolate as their working contemporaries to determine whether work has any effect on the parasitaemia pattern observed.

This study indicated that if surra does depress the work output of buffalo the magnitude of any fall may vary considerably depending upon the strain of the parasite with which the animals become infected. The six group 1 buffalo infected with Bakit 362 worked normally for 5 weeks with no signs of a decline in output, unlike the animals in group 2 infected with Garut 197. Such variations may be due to strain differences in infectivity and pathogenicity, possibly linked to differences in the virulence of the parasite. The implications of this for draught animals in the field require further investigation. The importance of host resistance to the parasite also requires further study; the buffalo used here were all young animals selected on the basis that they were parasitologically negative for *T. evansi* at the time of the study and did not have antibodies to the disease by ELISA. In areas of enzootic stability, surra may have little or no effect on work output particularly amongst older buffalo, unless novel, virulent strains of the parasite are imported. If surra is shown to depress work output under experimental conditions, large-scale field studies will be needed in Indonesia and elsewhere in S.E. Asia to assess its impact on farm productivity before practical cost-effective control measures can be devised. One way of achieving this would be to carry out performance tests involving fixed amounts of work on two groups of farmers' animals at strategic intervals during the working season, one group given prophylactic protection against surra, the other not. Both groups would be screened for trypanosomes at regular intervals. The success of the study would of course depend on a high proportion of the unprotected animals contracting surra. The two groups would have to be large because of the multitude of other variables which could affect output. This type of study does however raise serious ethical

questions since farmers whose animals became parasitaemic would not be told of this lest they worked those animals differently or immediately treated them with trypanocides. Some form of compensation might be required to reimburse farmers for any loss of productivity on their farms.

Surra is generally regarded as a chronic disease of cattle and buffalo in S.E. Asia. In this study work output was only monitored for two short periods (five and six weeks). If surra is subsequently proven to affect work output it may be necessary to examine the effects of the disease over longer periods, particularly in view of the apparent recovery observed in the outputs of the infected animals right at the end of the study. The work outputs of the teams in group 2 which had fallen rapidly with increasing parasitaemias in some of the animals, rose slightly on the last day of the study in concert with a fall in parasitaemias. In view of the fluctuations seen in work output over the trial it is obviously impossible to draw any conclusions from a single day's data, however even sporadic parasitaemic episodes depressing work output by up to 40% in ten days could have serious implications for the timeliness of cultivation operations in the field during short growing seasons.

When this study was originally designed it was assumed that all buffalo infected with *T. evansi* would develop patent parasitaemias. In retrospect it would have been sensible to infect more animals than were actually required to ensure that sufficient parasitaemic animals were available. Working parasitaemic and aparasitaemic buffalo together on the same yoke was not ideal. Unfortunately working animals singly was not a feasible option because of track size and lack of equipment.

This study clearly illustrated the large number of variables which can affect work performance and which consequently need to be monitored or controlled if changes in output are to be attributable to a specific cause.

The effects of exercise and plane of nutrition on sheep immune responses in the absence of disease

In this experiment moderate chronic exercise had no measurable effect on either humoral or cellular immune responses to non-pathogenic antigens in sheep. Undernutrition did have a minor effect on humoral responses to a T-lymphocyte activator; the speed of the primary antibody response to ovalbumin was slower in underfed animals than in those on an adequate plane of nutrition, however this was the only nutritional effect observed. It is possible that the stressors used were not severe enough to affect the immune responses measured, or that the techniques used were not sufficiently accurate to quantify any effects which did occur. Certainly in view of the evidence from other studies with other mammals (reviewed in sections 2.2.5 and 2.2.6), it seems unlikely that neither exercise nor undernutrition affects immunity in sheep.

The amount of exercise used in the trial was modest for a breed of sheep accustomed to living on the Scottish hills, although personal observations suggested that the animals were stressed by it. The stress was probably due to the speed of walking and the continuous nature of the exercise, rather than the overall distance travelled. The lower plane of nutrition did cause the animals to lose significant amounts of weight, but the losses were proportionally much smaller than those often incurred by working animals on farms. Welfare considerations together with practical constraints precluded more strenuous exercise and more extreme nutritional restrictions which might have had a measurable effect on immune responses. Giving a large number of sheep a controlled amount of exercise over several hours is not easy, although the horsewalker used here proved adequate for the task.

The results of this study were disappointing in that the techniques employed to assess immune responses had been successfully used in a previous experiment to demonstrate immunosuppression in sheep infected with *A. variegatum* ticks (Lloyd

and Walker, 1993). The absence of significant differences in humoral and cellular responses between the four groups of animals may have been because the stressors used were not severe enough, or because neither plane of nutrition nor exercise affect sheep immune responses, however it is also possible that the immunosuppression caused by adult ticks differs from that induced by exercise or undernutrition.

Both humoral and cellular responses varied considerably between individuals within the four groups of sheep making it difficult to detect differences between groups. In view of the antigens used and the assay quality control measures adopted for the ELISA programme it seems likely that the large variations in primary humoral responses observed within groups were attributable to differences in individual responsiveness. The variations in cellular responses within groups were almost certainly due to a combination of differences in individual responsiveness and inaccuracies associated with the skin testing procedure. Fiske and Adams (1985) studying the effects of malnutrition on immunity in cattle suggested that variations in individual responsiveness might be minimised by using groups of animals from a very restricted genetic background. Nothing was known of the genetic background of these sheep.

This experiment did provide a good test of assay techniques for assessing humoral and cell mediated immunity in exercised and rested animals under controlled conditions, before further studies were undertaken in the tropics. The ELISA technology worked well once certain quality control steps were introduced to minimise plate to plate and day to day variations in results. In contrast skin tests were difficult to administer and results obtained within groups and on individual animals were very variable. The skin testing procedure would have been simplified by the use of a semi-automated intradermal injector. With hindsight however skin tests are probably only useful in studies with few animals. The technique is good for determining positive or negative responses to an antigen but it does not appear to be

sufficiently sensitive to quantify responses accurately. Before any future investigations on the effects of exercise stress on cell mediated immunity are undertaken alternative assay techniques such as the use of FACS analysis must be explored.

Using sheep as a model to give indications of the likely effects of exercise and undernutrition on immune responses in larger ruminants is a valid technique. As this study has demonstrated however, it is very difficult to judge how much exercise to give the animals to simulate draught animals working on farms. Welfare considerations in the UK mean that the stressors which many draught animals routinely experience in the tropics are unacceptable here.

The effects of work and undernutrition on the trypanotolerance of N'Dama cattle

Trypanotolerant cattle such as the N'Dama are able to survive and recover from trypanosome infections without the aid of trypanocidal drugs, however whilst infected they often suffer from a loss of productivity including reduced growth rates, poor milk yields and impaired reproductive performance. This trial has clearly demonstrated for the first time that the work performance of N'Dama used for draught power may also be markedly reduced by trypanosome infections and furthermore that working infected animals can adversely affect their tolerance of such infections. Whilst any fall in the productivity of a farmer's animals is unwelcome, the time dependent nature of most cultivation operations means that a fall in the work output of draught animals can have particularly serious and far reaching consequences for farm productivity.

The work demanded of the cattle in this study was not arduous compared to that normally expected of farmers' animals in The Gambia and elsewhere, yet speeds fell as soon as the animals became parasitaemic. Within four weeks of infection the

working speeds of some teams had almost halved, the mean reduction in speed was 30%, resulting in a similar loss of power. With heavier workloads on farms, the work output of N'Dama infected with trypanosomes might decline more rapidly with speeds falling even lower. Similarly, more virulent or more pathogenic strains and species of trypanosome might also cause a more rapid on more pronounced fall in work performance. Further trials are required to investigate the effects of different workloads and different trypanosomes on the work performance of N'Dama.

Possibly of greater importance to farmers than the decline in working speeds observed in this experiment, was the failure of five of the eight infected teams to complete the 8 weeks of work post-infection. The first team had to be retired less than four weeks after the bulls were infected. This suggests that some N'Dama infected with *T. congolense* may not be able to sustain even light work for long periods. In tsetse fly infested areas farmers wishing to work their N'Dama for more than 2 to 3 weeks without prophylactic protection against trypanosomosis may need to keep plenty of spare animals to ensure that they have sufficient animals to complete the tasks required of them.

In this study teams were withdrawn from work when the blood PCV of one or other of the bulls fell to a critical level below which the animal's life was considered to be at risk. Using this technique all the animals survived the experiment without the aid of trypanocidal drugs, however it is not a practical technique for farmers seeking to work N'Dama harbouring trypanosome infections. Farmers without access to laboratory facilities and trained personnel capable of monitoring blood PCV should beware of working infected animals for too long in order to try to complete urgent cultivation tasks, lest it has fatal consequences for the animals. The loss of a draught animal is a major catastrophe for most farmers because of the time required to train a replacement and because good, trained, animals are seldom sold. The effects of work on the ability of N'Dama to recover from trypanosome infections without the aid of

drugs, were not considered as part of this experiment; all animals were treated with a trypanocide at the end of the 12 week work period. Until such studies are undertaken, advice to farmers working animals they know to be infected with trypanosomes must be to proceed with caution and to cease working the animals as soon as possible.

Trypanosomosis did not stop all the N'Dama in this trial from working suggesting that as with other production traits, individual variations in trypanotolerance may determine whether or not a draught animal is able to continue working whilst infected with trypanosomes. The work performance of the three teams which were able to continue working until the end of the study actually started to recover five weeks after infection once the bulls had passed the point of peak parasitaemias. Unfortunately the short duration of the study meant that it was not possible to determine whether this recovery was sustainable. Further trials are required to investigate whether N'Dama that work through the first parasitaemic episodes of trypanosome infections are then able to continue working without any further adverse effects on performance or whether work output continues to fluctuate in concert with subsequent waves of parasitaemia. Assuming that some animals are able to continue working despite being infected with trypanosomes, the problem is how to identify those individuals possessing this superior tolerance so that they can be selected as draught animals.

One of the common symptoms of trypanosomosis is a loss of body condition caused by a depression in appetite, possibly exacerbated by an increase in the animal's maintenance requirements and a reduction in the efficiency of nutrient utilisation. Results from this study suggest that it may be difficult if not impossible for farmers to stem such weight losses in working animals regardless of the feedstuffs available to them, because of the extra energy demands imposed on animals for work and because work may also adversely affect the efficiency of nutrient utilisation. In the field the

main working season for draught animals is normally during and immediately after the rains when feed is most scarce following the dry season. Unfortunately such moist, damp conditions are ideal for tsetse fly breeding. In animals which are able to continue working despite trypanosome infections pronounced weight losses may reduce work output. Ultimately unless such weight losses are halted the animals may become too weak to work. Further studies need to be conducted to explore the effects of both trypanosome infections and work on nutrient metabolism in N'Dama cattle.

The majority of the animals used in this study were immunologically naive with respect to the trypanosome isolate with which they were infected. In areas where trypanosomosis is endemic the disease may have a much smaller effect on the work output of older N'Dama with prior experience of infection, provided that such animals do not become infected with a novel isolate of the parasite which they have not previously encountered. The effects of trypanosomosis on the work output of farmers' animals need to be quantified in the field.

In addition to the effect of trypanosomosis on work output, this experiment has clearly demonstrated that work stress can significantly reduce the trypanotolerance of N'Dama cattle, causing them to develop higher parasitaemias and more severe anaemias than animals which are not worked. Thus although it may be possible to keep N'Dama for beef and milk production in tsetse fly infested areas without the use of trypanocides albeit with some loss of productivity, it may not be feasible to use those animals to provide draught power without protecting them against trypanosomosis. How work affects trypanotolerance is not clear. Initial observations from this study suggest that it does not affect humoral immune responses to the disease. Further trials are needed to determine whether work has any effect on cellular immune responses, however until the exact mechanisms behind

trypanotolerance itself are elucidated it is difficult to know which components of the immune response to examine.

General conclusions

The experiments described in this thesis have clearly demonstrated that under controlled conditions disease can markedly reduce the work output of draught cattle within a short time of the animals becoming infected. They have also shown that work can reduce the natural resistance of N'Dama to trypanosomosis causing them to develop high parasitaemias and severe, life threatening anaemias. The adverse effects of work on trypanotolerance do not appear to be mitigated by plane of nutrition. It has not however been possible to elucidate how work stress might affect immune responses to a disease challenge. The studies have clearly illustrated the large number of variables that can affect both work performance and immunity in individual animals and the consequent difficulty of proving cause and effect.

Further field studies are required to quantify the impact of different diseases on the work performance of draught animals in different locations before appropriate cost effective control measures can be devised for farmers. Because of their geographical spread affecting animals world-wide and because infections often go undetected for months even years, priority should be given to an examination of the effects of gastrointestinal parasites on the work performance of draught animals. To determine how the work done by draught animals might affect their immune responses further laboratory based experiments are needed employing a variety of non-pathogenic immunomodulators to stimulate different components of the immune system. These experiments should be conducted under strictly controlled conditions with groups of genetically related animals all of which have the same immunological background. Suitable facilities would be required to give large animals fixed amounts of exercise over long periods of time.

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9. APPENDICES

1. Composition of PBS/Tween 20, ELISA washing solution.

Sodium chloride	8.0 g
Potassium dihydrogen orthophosphate	0.2 g
Sodium hydrogen orthophosphate dodecahydrate	2.9 g
Potassium chloride	0.2 g
Tween 20	0.5 ml

2. Transformation of ELISA OD data to compare duplicates.

OD	anti-$\log_{10}$	10 % of the anti-$\log_{10}$	Acceptable OD range
0.1	1.2589	0.1259	0.05 – 0.14
0.9	7.9433	0.7943	0.85 – 0.94

3. Positive and negative control OD values for the Sheep study (Chapter 5), Ovalbumin ELISA, with binding ratios and F values.

Plate 20 was used as "plate 0" for calculation of the F factor correction.

Plate no.	Samples tested (day)	F value	Positive control	Negative control	PBS blank	Binding ratio
1*	day 11 & 78	1.00	1.3132	0.1178	0.0527	19
2	32 & 53	1.04	1.2026	0.0574	0.0484	128
3	60 & 64	1.11	1.1490	0.0706	0.0630	142
4	67 & 71	1.10	1.1407	0.0593	0.0502	119
5	74 & 78	1.06	1.1927	0.0638	0.0523	99
6	81 & 85	0.93	1.3517	0.0643	0.0519	105
7	88 & 92	1.00	1.2647	0.0680	0.0526	79
8	95 & 99	1.30	0.9867	0.0710	0.0512	47
9	102 & 106	0.99	1.2880	0.0832	0.0673	77
10	109 & 113	0.90	1.4147	0.0916	0.0600	43
11	116 & 120	0.99	1.2767	0.0766	0.0598	73
12	123 & 127	0.88	1.4412	0.0918	0.0623	47
13	130 & 150	1.07	1.2015	0.0913	0.0528	30
14	11, 32 & 53	1.42	0.8952	0.0580	0.0695	infinity
15*	60,64 & 67	1.48	0.8860	0.0782	0.0720	133
16	71, 74 & 78	1.04	1.2148	0.0710	0.0480	51
17	81, 99 & 102	1.01	1.2807	0.0960	0.0750	57
18	85, 88 & 92	1.20	1.0610	0.0638	0.0530	93
19	95, 106 & 109	0.88	1.4514	0.0966	0.0705	53
20	113, 116 & 120	1.00	1.2698	0.0772	0.0587	65
21	123, 127 & 130	0.92	1.3578	0.0684	0.0642	309
22	misc. rpts.	1.23	1.0260	0.0574	0.0478	102
23	150 & misc rpts	0.86	1.4777	0.0836	0.0629	68
24	misc. rpts.	1.07	1.1723	0.0570	0.0478	123
25	misc. rpts.	0.97	1.3075	0.0800	0.0518	44
26	misc. rpts.	1.16	1.1077	0.0800	0.0556	43
27	misc. rpts.	0.83	1.5132	0.0788	0.0510	53
28	misc. rpts.	0.84	1.5018	0.0847	0.0548	48
29*	misc. rpts.	0.69	1.8217	0.1036	0.0506	33
30	misc. rpts.	1.03	1.2487	0.0884	0.0658	52
32	misc. rpts.	1.01	1.2405	0.0540	0.0455	140
33	misc. rpts.	0.89	1.4257	0.0805	0.0613	71
34	misc. rpts.	0.94	1.3480	0.0828	0.0633	66
Mean			1.2676	0.0772	0.0575	
s.d.			0.18957	0.01505	0.00812	
s.e.			0.03300	0.00262	0.00144	
c.v. (%)			15%	19%	14%	

* Plates with positive or negative controls that differ from the mean by more two standard deviations.

4. Positive and negative control OD values for the Sheep study (Chapter 5), *B. abortus* ELISA, with binding ratios and F values.

Plate 13 was used as "plate 0" for calculation of the F factor correction.

Plate	Samples tested	F value	Positive control	Negative control	PBS blank	Binding ratio
1	day 11 & 78	1.26	1.0744	0.0710	0.0518	53
2	32 & 53	1.06	1.2525	0.0587	0.0513	163
3	60 & 64	1.12	1.2010	0.0656	0.0531	92
4	67 & 71	1.27	1.0607	0.0638	0.0617	461
5	74 & 78	1.02	1.3000	0.0600	0.0562	327
6	81 & 85	1.03	1.2942	0.0633	0.0504	96
7	88 & 92	1.02	1.3052	0.0624	0.0550	169
8	95 & 99	1.17	1.1482	0.0672	0.0536	81
9	102 & 106	0.99	1.3748	0.0948	0.0774	75
10	109 & 113	0.90	1.4913	0.0835	0.0660	81
11	116 & 120	0.98	1.3830	0.0835	0.0737	133
12	123 & 127	0.89	1.5055	0.0795	0.0682	127
13	130 & 150	1.00	1.3487	0.0810	0.0622	68
14	11, 32 & 53	1.07	1.2387	0.0585	0.0530	214
15	60,64 & 67	1.06	1.2802	0.0883	0.0847	325
16	71, 74 & 78	1.06	1.2552	0.0557	0.0551	2100
17	81, 99 & 102	1.07	1.2827	0.1008	0.1189	infinity
18	85, 88 & 92	0.93	1.4350	0.0743	0.0687	244
19*	95, 106 & 109	0.95	1.4880	0.1496	0.0623	16
20	113, 116 & 120	0.93	1.4220	0.0653	0.0580	187
21	123, 127 & 130	0.87	1.5350	0.0778	0.0563	69
22	39 & 46	1.10	1.2130	0.0616	0.0479	85
23*	misc. rpts.	0.83	1.6083	0.0857	0.0667	81
24	misc. rpts.	1.06	1.2480	0.0524	0.0487	321
25	misc. rpts.	0.97	1.3875	0.0825	0.0750	175
Mean			1.3253	0.0755	0.0630	
s.d.			0.13907	0.02008	0.01517	
s.e.			0.02781	0.00402	0.00303	
c.v. (%)			10%	27%	24%	

* Plates with positive or negative controls that differ from the mean by more two standard deviations.

5. Positive and negative control OD values for the N'Dama cattle study (Chapter 6) IgG_1 ELISA with binding ratios and F values.

Plate 20 used as F factor correction plate as it is nearest to the mean positive & negative controls.

Plate	Date tested	Samples tested	F value	Positive control	Negative control	PBS blank	Binding ratio
1	11/07/94	day 29	0.59	0.511	0.059	0.047	37
2 *	11/07/94	day 31	0.55	0.556	0.069	0.045	21
3 *	11/07/94	day 33	0.56	0.555	0.082	0.061	23
4 *	11/07/94	day 36	0.53	0.598	0.099	0.069	17
5	13/07/94	day 38	1.14	0.288	0.054	0.045	27
6	13/07/94	day 40	1.23	0.276	0.059	0.055	59
7	14/07/94	day 43	0.95	0.341	0.059	0.045	20
8	14/07/94	day 45	0.94	0.345	0.062	0.045	17
9	14/07/94	day 47	1.93	0.188	0.050	0.047	51
10	14/07/94	day 50	1.70	0.205	0.049	0.042	25
11	15/07/94	day 52	1.33	0.253	0.052	0.047	46
12	15/07/94	day 54	1.83	0.195	0.050	0.045	30
13	15/07/94	day 57	1.85	0.193	0.049	0.045	37
14	15/07/94	day 59	1.48	0.228	0.048	0.047	361
15	18/07/94	day 61	0.78	0.423	0.081	0.049	11
16	18/07/94	day 64	0.99	0.325	0.055	0.046	31
17	18/07/94	day 66	0.73	0.426	0.062	0.056	59
18	18/07/94	day 68	0.71	0.446	0.073	0.058	27
19	19/07/94	day 71	0.90	0.354	0.058	0.045	25
20	19/07/94	day 73	1.00	0.324	0.058	0.047	25
21	19/07/94	day 75	0.90	0.358	0.063	0.051	26
22	19/07/94	day 78	0.88	0.366	0.062	0.052	32
23	20/07/94	day 80	0.91	0.349	0.058	0.043	21
24	20/07/94	day 82	1.00	0.326	0.059	0.060	infinity
25	20/07/94	day 85	1.21	0.280	0.060	0.052	30
26	28/07/94	repeats	2.39	0.163	0.051	0.046	25
28	29/07/94	repeats	1.20	0.269	0.047	0.040	37
30	29/07/94	day 24	2.00	0.177	0.043	0.044	infinity
33	10/10/94	day 1	1.24	0.261	0.047	0.048	261
34	10/10/94	day 3	1.02	0.314	0.051	0.045	45
35	10/10/94	day 5	1.19	0.276	0.051	0.043	27
36	10/10/94	Pre-expt	1.79	0.198	0.049	0.048	100
37	10/10/94	day 26	1.50	0.221	0.042	0.043	infinity
Mean				0.3208	0.0578	0.0484	
s.d.				0.11550	0.01208	0.00627	
s.e.				0.02011	0.00210	0.00109	
c.v. (%)				36	21	13	

* Plates with positive or negative controls that differ from the mean by more than 2 s.d.

6. Positive and negative control OD values for the N'Dama cattle study (Chapter 6) IgM ELISA with binding ratios and F values.

Plate 24 used as F factor correction plate as it is nearest to the mean positive & negative controls.

Plate	Date tested	Samples tested	F value	Positive control	Negative control	PBS blank	Binding ratio
1 *	09/09/94	day 29	0.64	0.594	0.240	0.080	3
2	09/09/94	day 31	0.68	0.506	0.174	0.083	5
3	09/09/94	day 33	0.74	0.486	0.181	0.066	4
4	09/09/94	day 36	0.80	0.453	0.172	0.085	4
5	10/09/94	day 38	1.39	0.297	0.136	0.080	4
6	10/09/94	day 40	1.02	0.368	0.149	0.073	4
7	10/09/94	day 43	1.13	0.346	0.147	0.060	3
8	10/09/94	day 45	0.90	0.406	0.155	0.071	4
9	11/09/94	day 47	1.05	0.384	0.170	0.069	3
10	11/09/94	day 50	0.81	0.438	0.162	0.066	4
11	11/09/94	day 52	2.03	0.238	0.127	0.064	3
12	11/09/94	day 54	1.44	0.287	0.131	0.067	3
13	11/09/94	day 57	1.71	0.221	0.090	0.048	4
14	11/09/94	day 59	1.44	0.258	0.101	0.077	7
15	12/09/94	day 61	1.33	0.362	0.193	0.073	2
16	12/09/94	day 64	0.90	0.429	0.179	0.078	3
17	12/09/94	day 66	1.71	0.239	0.108	0.059	4
18	12/09/94	day 68	1.46	0.292	0.138	0.096	5
19	13/09/94	day 71	0.77	0.419	0.128	0.051	5
20	13/09/94	day 73	0.79	0.458	0.175	0.068	4
21	13/09/94	day 75	1.10	0.311	0.107	0.052	5
22	13/09/94	day 78	0.94	0.362	0.122	0.052	4
23	14/09/94	day 80	1.18	0.326	0.135	0.087	5
24	14/09/94	day 82	1.00	0.361	0.136	0.081	5
25	14/09/94	day 85	1.46	0.259	0.105	0.068	5
26	14/09/94	day 26	1.30	0.296	0.123	0.070	4
27	15/09/94	day 24	0.89	0.410	0.158	0.063	4
31	23/09/94	Pre-expt	0.89	0.376	0.123	0.054	5
32	23/09/94	day 1	1.07	0.347	0.138	0.055	4
33	23/09/94	day 3	1.32	0.269	0.098	0.049	4
34	23/09/94	day 5	1.03	0.371	0.152	0.060	3
Mean				0.3604	0.1436	0.0679	
s.d.				0.08745	0.03242	0.01235	
s.e.				0.01571	0.00582	0.00222	
c.v. (%)				24	23	18	

* Plate with positive & negative controls that differ from the means by more than 2 s.d.

7. Positive and negative control OD values for the N'Dama cattle study (Chapter 6) IgG ELISA with binding ratios and F values.

Plate 34 used as F factor correction plate as it is nearest to the mean positive & negative controls.

Plate	Date tested	Samples tested	F value	Positive control	Negative control	PBS blank	Binding ratio
7	27/09/94	day 29	0.87	0.473	0.070	0.053	25
8	27/09/94	day 31	0.88	0.469	0.071	0.055	27
9	27/09/94	day 33	0.77	0.520	0.065	0.052	35
10	27/09/94	day 36	1.04	0.415	0.078	0.056	16
11	27/09/94	day 38	0.91	0.452	0.068	0.049	21
12	27/09/94	day 40	0.93	0.449	0.071	0.060	35
13	28/09/94	day 43	1.23	0.372	0.088	0.079	33
14	28/09/94	day 45	0.94	0.437	0.062	0.044	22
15	28/09/94	day 47	0.87	0.467	0.063	0.047	28
16	28/09/94	day 50	0.85	0.497	0.083	0.059	18
17	28/09/94	day 52	0.83	0.507	0.084	0.051	14
18	28/09/94	day 54	0.79	0.508	0.064	0.047	28
19	29/09/94	day 57	0.88	0.455	0.056	0.043	32
20	29/09/94	day 59	0.93	0.435	0.059	0.045	27
21	29/09/94	day 61	0.89	0.452	0.059	0.045	30
22	29/09/94	day 64	0.87	0.462	0.059	0.049	40
23 *	29/09/94	day 66	1.26	0.332	0.054	0.041	21
24	29/09/94	day 68	1.18	0.359	0.063	0.054	33
25	30/09/94	day 71	0.80	0.565	0.124	0.119	105
26	30/09/94	day 73	0.76	0.599	0.138	0.128	46
27	30/09/94	day 75	0.76	0.602	0.141	0.127	36
28	30/09/94	day 78	0.78	0.580	0.130	0.118	38
29	30/09/94	day 80	0.75	0.612	0.148	0.128	25
30	30/09/94	day 82	0.80	0.607	0.166	0.127	12
31	01/10/94	day 85	1.01	0.481	0.136	0.117	19
32	01/10/94	Pre-expt	0.92	0.511	0.131	0.120	36
33	01/10/94	day 26	0.87	0.537	0.134	0.116	23
34	01/10/94	day 1	1.00	0.489	0.138	0.138	702
35	01/10/94	day 3	0.98	0.496	0.138	0.118	19
36	01/10/94	day 5	1.02	0.503	0.159	0.138	17
37	06/10/94	day 24	1.00	0.430	0.081	0.045	11
Mean				0.4862	0.0960	0.0795	
s.d.				0.07059	0.03724	0.03720	
s.e.				0.01268	0.00669	0.00668	
c.v. (%)				15	39	47	

* Plate with positive control that differs from the mean by more than 2 s.d.

8. Kruskal-Wallis test of mean parasitaemia scores with a multiple comparison test to determine which groups were significantly different (using data from the N'Dama cattle study, Chapter 6).

Kruskal-Wallis Test

LEVEL	NOBS	MEDIAN	AVE. RANK	Z VALUE
BW	8	3.579	20.3	1.33
BN	8	3.000	8.1	-2.94
HW	8	3.628	21.8	1.83
HN	8	3.440	15.9	-0.22
OVERALL	32		16.5	

H = 10.33 d.f. = 3 p = 0.016
H = 10.36 d.f. = 3 p = 0.016 (adjusted for ties) *significant difference* *

Multiple comparisons between means (Siegal and Castellan, 1989)

k = no. of groups = 4
n = no. of observations per group = 8
N = total no. of observations = 32
c = k(k-1)/2 = 6
α = required probability level = 0.05
$Z_{(\alpha/k(k-1))}$ = the abscissa value from the unit normal distribution above which lies $\alpha/k(k-1)$ percent of the distribution, obtained from modified normal distribution tables.
$Z_{(c=6,\ \alpha=0.05)} = 2.638$

Critical difference between ranks = $Z\sqrt{(N(N+1)/12)(1/n_1+1/n_2)} = 12.37$

Differences between ranks:

HW - BN = 21.8 - 8.1 = 13.7	*significantly different* *
BW - BN = 20.3 - 8.1 = 12.2	*not significantly different*
HN - BN = 15.9 - 8.1 = 7.8	*not significantly different*
HW - BW = 21.8 - 20.3 = 1.5	*not significantly different*
HW - HN = 21.8 - 15.9 = 5.9	*not significantly different*
BW - HN = 20.3 - 15.9 = 4.4	*not significantly different*

9. Calculation of log equivalent parasitaemias, after Walker (1969).

LEV = (Log10 (no. of trypanosomes seen/no. of fields examined)) + 2

No. of trypanosomes counted / field by DG technique	Log equivalent value
MHCT - ve	0.00 +
MHCT + ve, 0 trypanosomes / 20 fields	0.25 +
1 trypanosomes / 20 fields	0.70
1 trypanosomes / 1 field	2.00
10 trypanosomes / 1 field	3.00
20 trypanosomes / 1 fields	3.30
100 trypanosomes / 1 field	4.00
> 100 trypanosomes / 1 field	5.00 +

+ Indicates arbitrary values

10. LEV parasitaemias for each animal in group 1 on each sampling occasion (Indonesian study, Chapter 4).

Blanks indicate no trypanosomes detected.
The shaded area indicates when animals were not being worked.

Animal		402		409		400		404	
Time (hh:mm)		06:30	11:00	06:30	11:00	06:30	11:00	06:30	11:00
Expt. day	Days p.i.								
0	1								
1	2						1.00	0.25	0.25
2	3				1.18		1.00	1.54	1.74
3	4			2.19	1.30	1.40	1.48	2.10	2.42
4	5			3.40	3.70	0.25	0.25	2.38	0.25
5	6			1.78	1.54		0.25		
6	7			0.25					
7	8			0.25				0.25	
8	9			3.51	3.64	0.70		1.40	2.33
9	10			3.70	4.00	0.25	0.25	3.02	3.33
10	11	0.25		5.00		0.25		3.52	
11	12	1.74	2.00	5.00	5.00	2.56	2.47	4.00	5.00
12	13	2.40	2.92	5.00	5.00	1.60	0.25	5.00	5.00
13	14			3.13		1.60		2.57	
14	15			4.00		0.70		3.40	
15	16			3.45	2.61	1.81	1.90	3.22	2.49
16	17	0.25	0.25	1.48	1.18	2.50	3.04	2.90	3.18
17	18			0.25		1.40		1.18	
18	19			0.25	0.25	1.00	0.25	1.54	1.00
19	20			0.25	1.18			0.25	0.70
20	21			3.34		0.25		1.54	
21	22			5.00		0.70		1.40	
22	23			5.00	3.70	0.70	0.25	1.81	1.48
23	24			3.70	3.13	0.25	0.70		
24	25			2.67	2.58	0.25	1.18		
25	26			2.88	3.07	0.25	0.25	0.25	0.25
26	27			1.30		0.25	1.18	0.25	0.25
27	28	0.25		0.25		1.00		1.00	
28	29	0.70				2.95		1.74	
29	30	1.00	1.70			3.70	4.00	3.30	3.36
30	31					0.70	0.25	2.00	2.10
31	32						0.25		
32	33								
33	34								
34	35								
35	36								
36	37	0.25							
37	38	0.25							
38	39	0.25				1.00			
39	40	0.25				1.78			
40	41					2.39			

11. LEV parasitaemias for each animal in group 2 on each sampling occasion (Indonesian study, Chapter 4).

Blanks indicate no trypanosomes detected. The shaded area indicates when the animals were not worki

Animal:		406		408		403		411		401		407	
Time (hh:mm):		06:30	11:00	06:30	11:00	06:30	11:00	06:30	11:00	06:30	11:00	06:30	11:00
Expt. day	Days p.i.												
35	1					0.25		2.20		0.25			
36	2	0.25				1.78		2.71		2.39			
37	3	1.60		0.25		3.33		3.70		5.00			
38	4	3.40		2.79		4.00		5.00		5.00			
39	5	3.52		3.32		4.00		3.45		3.01		0.25	
40	6	5.00		2.59		5.00		3.03		2.53		2.45	
41	7	2.34		2.78		4.00		3.70		3.10		2.86	
42	8	1.95		3.30		5.00		5.00		4.00		4.00	
43	9			3.02		3.70		3.70		5.00		3.68	
44	10			1.30		4.00		3.70		3.70			
45	11	0.25				3.70		4.00		3.70			
46	12					2.98		3.01		3.00			
47	13			0.25		1.85		2.63		0.25			
48	14			0.70		0.25		0.70		0.25			
49	15	0.25				0.70		1.48		2.18			
50	16	0.25	1.40	0.25			1.48		0.70	0.25	0.25		
51	17	2.63	3.14			1.30	0.25	0.70	0.25	0.25	0.25		
52	18	3.40	3.59	1.40	0.70	0.25	0.25	0.25	0.25	0.70	0.25		
53	19		0.25	1.60	0.25			0.25	0.25	0.25	0.25		
54	20						0.25		0.25	0.25			
55	21									1.18			
56	22												
57	23								0.25	0.25	0.25		
58	24						0.25	0.25		0.25	0.25		
59	25	0.25		0.25		0.25		0.25	0.25	0.25			
60	26	1.70	1.60	0.25	1.00	1.00	1.60	1.30	1.65	0.70	0.70		
61	27	1.65	1.30	0.25		1.30	0.25	0.70	0.25	0.25			
62	28	1.00				0.25		0.25					
63	29	0.25				0.25		0.25		0.25			
64	30	0.70	1.00	0.25	1.00	1.60	1.54	1.48	1.60	0.25	0.25		
65	31	1.00	1.60	0.25	0.25	2.32	2.90	0.25	1.00	1.65	0.25		
66	32	1.40	2.00	0.25	0.25	1.30	1.00	2.20	2.50	2.72	1.95		
67	33	0.25	0.25			2.11	1.70	0.70	1.30	3.49	3.70		
68	34	0.25	1.30		1.54	0.25		1.60	1.30	1.81	1.30		0.25
69	35					0.70		0.25		1.00			
70	36							0.25		2.37			
71	37		0.25					0.25	0.25	0.25	0.25		
72	38	0.25	0.25			0.25	0.70	0.25	0.25	1.74	1.88		
73	39	0.25	0.25			0.70	0.25	0.25	1.48	1.88	2.04		
74	40	0.25	1.18			0.25	0.70	0.25	1.00	1.00	1.48		
75	41	0.70	0.25	0.25	0.25	0.25	1.00	0.25	0.25	1.48	0.25		
76	42	0.25		0.25		0.25		0.25		0.25			
77	43			1.74		1.00		0.25		0.25			
78	44			1.00	0.25	0.25	0.25		0.25		0.25		
79	45		0.25		0.25	0.25	0.70	0.25	1.30	0.25	1.00		
80	46	1.60	2.49				0.25	2.84	3.19	2.20	2.53		
81	47	3.47	3.32			0.25	0.25	3.70	4.00	3.70	4.00		
82	48							3.70	4.00	4.00	2.72		
83	49					0.25		3.58		2.69			
84	50					0.25		3.74		3.70			
85	51				0.25	1.30	1.65	0.25	0.25	2.24	1.54		
86	52					2.64	3.03	1.00	1.00	0.25	1.30		
87	53					3.26	3.50	1.00	0.70	0.70	0.25		
88	54					2.24	2.22	0.25	1.00	0.25	0.25		
89	55	0.70	0.25			3.10	3.40	0.25	1.00	0.25	0.25		
90	56	1.90				0.70		0.70		0.25			
91	57												
92	58												

12. The ADF required by the blue buffalo team to pull its sledge each day (Indonesian study, Chapter 4).

Period 1		Period 2	
Day	**ADF (N)**	**Day**	**ADF (N)**
1	1028	50	970
2	695	51	1071
3	813	52	964
4	924	53	1040
5	893	54	724
8	755	57	783
9	–	58	826
11	789	59	1010
12	730	60	989
15	779	61	990
16	912	64	1044
18	575	65	976
19	665	66	869
22	809	67	976
23	895	68	863
24	931	71	738
25	850	72	900
26	321	74	
30	683	75	808
31	710	78	795
32	722	79	837
33	775	80	836
		81	882
		82	881
		85	889
		86	898
		87	938
		88	1001
		89	968
Mean	774.1		909.5
s.e.	32.68		17.77
Mean as % of team LW at start of period	11.2		12.2
n	21		28

13. ADF test day results for each buffalo team for each period (Indonesian study, Chapter 4).

Team	Period 1 ADF test					Period 2 ADF test				
	Team LW day –1 (kg)	Load pulled as % of team LW (%)	ADF mean ± s.e. (N)	ADF as % team LW on day –1 (%)	n	Team LW day 48 (kg)	Load pulled as % of team LW (%)	ADF mean ± s.e. (N)	ADF as % team LW on day 48 (%)	n
Group 1										
White	578	25.1	543 ± 12.5	9.6	13	658	25.2	774 ± 14.0	12.0	15
Red	610	25.2	680 ± 13.6	11.4	9	650	25.1	800 ± 10.2	12.5	15
Yellow	644	25.1	657 ± 15.9	10.4	8	704	25.4	855 ± 9.8	12.4	18
Group 2										
Green	506	24.7	523 ± 7.9	10.5	8	584	24.3	756 ± 7.9	13.2	15
Stripes	584	26.0	554 ± 6.6	9.7	10	654	25.1	794 ± 5.9	12.4	17
Blue	694	25.8	639 ± 10.8	9.4	12	748	25.0	834 ± 21.4	11.4	10

14. Weekly mean work output (MJ/day) for each buffalo team in each period (Indonesian study, Chapter 4).

Group 1

		White			Red			Yellow		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	3887	317	5	4289	392	5	4206	330	5
	2	4628	404	3	5526	362	3	5531	443	3
	3	3908	397	4	4472	508	4	3939	565	4
	4	4478	667	5	5081	770	5	4773	675	5
	5	3866	135	4	4629	223	3	3474	1018	4
2	1	3636	259	5	4650	274	5	3181	470	4
	2	4771	356	5	4622	436	5	4491	490	5
	3	5294	230	5	5316	206	5	5347	306	5
	4	5554	222	3	5725	228	2	5612	213	3
	5	4740	220	5	4865	162	5	4706	182	5
	6	4147	291	5	4348	329	5	3594	535	5

Group 2

		Green			Stripes			Blue		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	3569	275	5	3778	364	5	3690	383	5
	2	4341	376	4	4520	481	4	4808	395	4
	3	3691	372	4	3362	343	4	3968	236	4
	4	4154	6006	5	4020	591	5	4619	631	5
	5	3883	160	4	3942	169	4	3909	358	4
2	1	4385	269	5	4494	339	5	4756	266	5
	2	4437	334	5	4319	382	5	4715	483	5
	3	5027	205	5	4867	279	5	5589	234	5
	4	4818	462	4	5346	196	4	5636	287	4
	5	4742	147	5	4736	141	5	4307	326	5
	6	4055	305	5	3276	307	5	3848	320	5

15. Weekly mean working times per day (seconds/day) for each buffalo team in each period (Indonsian study, Chapter 4).

Group 1

		White			Red			Yellow		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	7359	211	5	6570	336	5	6633	172	5
	2	8791	270	4	8226	257	4	8606	403	4
	3	8301	306	4	7585	424	4	7142	536	4
	4	8291	404	5	7652	561	5	7596	430	5
	5	8022	257	4	7613	358	3	5961	1679	4
2	1	7263	406	5	7116	377	5	4116	553	4
	2	7021	381	5	6580	386	5	5995	404	5
	3	7357	382	5	7247	430	5	6903	357	5
	4	9228	403	4	8938	478	3	8518	130	4
	5	8463	364	5	8270	264	5	7381	191	5
	6	8254	377	5	8271	418	5	6426	742	5

Group 2

		Green			Stripes			Blue		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	7039	136	5	7078	220	5	6580	322	5
	2	8464	323	4	8343	483	4	8399	429	4
	3	7955	373	4	7191	390	4	7769	135	4
	4	8085	480	5	7643	447	5	7817	386	5
	5	8356	310	4	8073	309	4	7625	4689	4
2	1	6820	397	5	6856	428	5	7015	248	5
	2	66187	379	5	6228	392	5	6616	379	5
	3	7149	357	5	6726	367	5	7369	315	5
	4	8097	863	4	8734	197	4	8874	236	4
	5	8561	295	5	8176	319	5	7529	390	5
	6	8139	366	5	6830	539	5	7716	232	5

16. Weekly mean distances walked per day (m/day) by each buffalo team in each period (Indonesian study, Chapter 4).

Group 1

		White			Red			Yellow		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	5248	272	5	4691	263	5	4694	242	5
	2	7271	380	4	6933	216	4	7018	293	4
	3	6311	421	4	5892	596	4	5352	790	4
	4	7008	461	5	6502	558	5	6241	475	5
	5	6311	299	4	6247	272	3	4771	1418	4
2	1	5289	341	5	5251	349	5	3066	490	4
	2	5577	288	5	5213	338	5	4714	390	5
	3	6037	274	5	5883	302	5	5500	296	5
	4	7276	385	4	7085	345	3	6798	195	4
	5	6032	301	5	5993	202	5	5419	298	5
	6	4787	426	5	4864	455	5	3772	624	5

Group 2

		Green			Stripes			Blue		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	4989	180	5	4972	315	5	4241	360	5
	2	6993	328	4	6791	423	4	6210	293	4
	3	6210	526	4	5402	634	4	5503	356	4
	4	6786	475	5	6200	477	5	6199	361	5
	5	6564	297	4	6286	338	4	5453	596	4
2	1	5117	367	5	5021	409	5	5021	220	5
	2	5308	323	5	4925	295	5	5098	356	5
	3	5864	287	5	5423	270	5	5922	219	5
	4	6439	723	4	6893	195	4	6845	193	4
	5	6166	215	5	5898	188	5	5112	455	5
	6	4787	452	5	3696	392	5	4136	429	5

17. Weekly median working speeds (ms^{-1}) for each buffalo team in each period (Indonesian study, Chapter 4).

Group 1

		White			Red			Yellow		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	0.69	0.030	5	0.70	0.020	5	0.70	0.033	5
	2	0.82	0.066	4	0.82	0.050	4	0.79	0.046	4
	3	0.78	0.056	4	0.78	0.079	4	0.77	0.108	4
	4	0.85	0.035	5	0.84	0.038	5	0.82	0.045	5
	5	0.78	0.045	4	0.79	0.035	3	0.79	0.103	4
2	1	0.73	0.025	5	0.75	0.033	5	0.75	0.070	4
	2	0.80	0.018	5	0.79	0.020	5	0.79	0.030	5
	3	0.83	0.015	5	0.81	0.020	5	0.79	0.025	5
	4	0.80	0.035	4	0.80	0.035	3	0.80	0.026	4
	5	0.71	0.075	5	0.74	0.073	5	0.76	0.073	5
	6	0.57	0.063	5	0.59	0.068	5	0.59	0.083	5

Group 2

		Green			Stripes			Blue		
Period	**Week**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**	**mean**	**s.e.**	**n**
1	1	0.76	0.035	5	0.69	0.048	5	0.63	0.043	5
	2	0.82	0.038	4	0.80	0.046	4	0.73	0.035	4
	3	0.79	0.060	4	0.76	0.114	4	0.74	0.059	4
	4	0.85	0.035	5	0.80	0.028	5	0.79	0.038	5
	5	0.77	0.046	4	0.77	0.046	4	0.74	0.083	4
2	1	0.76	0.025	5	0.72	0.033	5	0.72	0.023	5
	2	0.80	0.015	5	0.80	0.023	5	0.76	0.028	5
	3	0.82	0.015	5	0.81	0.025	5	0.81	0.023	5
	4	0.79	0.036	4	0.79	0.023	4	0.77	0.024	4
	5	0.73	0.078	5	0.74	0.075	5	0.65	0.085	5
	6	0.58	0.063	5	0.52	0.085	5	0.52	0.095	5

18. Estimated mean NE_{work} for each buffalo in each period per unit of metabolic liveweight and as a multiple of the animal's NE requirement for maintenance, with results of T-tests comparing period diferences (Indonesian study, Chapter 4).

Group1

		Estimated NE used for work						
		Period 1 ($KJ/kg^{0.75}$)			Period 2 ($KJ/kg^{0.75}$)			T-tests
Buffalo	Team	Mean	s.e.	n	Mean	s.e.	n	P_{period}
402	White	132.8	4.84	21	134.4	3.77	28	0.80
405	White	134.7	4.95	21	135.8	3.78	28	0.86
409	Red	140.9	6.06	20	138.3	3.88	27	0.70
410	Red	137.8	5.92	20	132.6	3.74	27	0.43
400	Yellow	133.8	6.10	20	120.8	6.18	27	0.15
404	Yellow	129.4	5.80	20	113.8	5.84	27	0.07
		Estimated NE_{work} as a multiple of $NE_{maintenance}$						
402	White	0.35	0.013	21	0.35	0.010	28	
405	White	0.35	0.013	21	0.36	0.010	28	
409	Red	0.37	0.016	20	0.36	0.010	27	
410	Red	0.36	0.015	20	0.35	0.010	27	
400	Yellow	0.35	0.016	20	0.32	0.016	27	
404	Yellow	0.34	0.015	20	0.30	0.015	27	

Group2

		Estimated NE used for work						
		Period 1 ($KJ/kg^{0.75}$)			Period 2 ($KJ/kg^{0.75}$)			T-tests
Buffalo	Team	Mean	s.e.	n	Mean	s.e.	n	P_{period}
406	Green	136.2	4.83	21	138.1	3.86	28	0.75
408	Green	132.3	4.67	21	132.2	3.68	28	0.99
403	Stripes	124.0	4.92	21	124.6	4.60	28	0.94
411	Stripes	124.7	4.98	21	123.1	4.56	28	0.82
401	Bue	120.0	4.86	21	125.0	4.52	28	0.46
407	Blue	117.0	4.74	21	121.8	4.41	28	0.47
		Estimated NE_{work} as a multiple of $NE_{maintenance}$						
406	Green	0.35	0.012	21	0.36	0.010	28	
408	Green	0.34	0.012	21	0.34	0.010	28	
403	Stripes	0.32	0.013	21	0.33	0.012	28	
411	Stripes	0.32	0.013	21	0.32	0.012	28	
401	Bue	0.31	0.013	21	0.33	0.012	28	
407	Blue	0.31	0.013	21	0.32	0.012	28	

19. Median buffalo body temperatures (°C) at 06.30h during each period with results of Mann-Whitney tests to compare period differences (Indonesian study, Chapter 4).

Group 1

	Period 1 (infected)			Period 2 (treated)			MW test		
Buffalo	Median	s.i.r.	n	Median	s.i.r.	n	W	P_{period}	Signif
402	37.8	0.18	25	38.0	0.25	40	718	0.15	ns
405 [$]	37.5	0.20	32	38.0	0.29	40	848	<0.001	***
409	38.0	0.33	32	38.0	0.24	40	1138	0.74	ns
410 [$]	38.0	0.24	32	38.0	0.15	40	994	0.045	*
400	37.9	0.19	32	38.0	0.15	40	1111	0.52	ns
404	38.1	0.19	32	38.0	0.15	40	1193	0.78	ns

[$] Buffalo that did not develop patent parasitaemias

Group 2

	Period 1 (not infect)			Period 2 (infected)			MW test		
Buffalo	Median	s.i.r.	n	Median	s.i.r.	n	W	P_{period}	Signif
406	37.7	0.30	32	38.2	0.29	40	834	<0.001	***
408	38.0	0.27	32	38.4	0.19	40	779	<0.001	***
403	37.7	0.20	32	37.9	0.15	40	904	0.003	**
411	38.0	0.20	32	38.3	0.25	40	855	<0.001	***
401	38.0	0.15	32	37.9	0.14	40	1161	0.94	ns
407	37.9	0.24	32	38.0	0.25	40	1076	0.30	ns

20. Median rates of body temperature change for each buffalo in group 1 over each work session in periods 1 and 2, with Mann-Whitney tests comparing period differences (Chapter 4).

		Rates of body temperature change (°C/min)						MW test	
	Work	Period 1 (infected)			Period 2 (treated)				
Buffalo	session	Median	s.i.r.	n	Median	s.i.r.	n	P_{period}	Signif
402	first	0.019	0.0087	22	0.017	0.0055	29	0.37	ns
	rest	0.000	0.0060	22	-0.005	0.0073	28	0.46	ns
	second	0.020	0.0064	22	0.019	0.0069	28	0.85	ns
405	first	0.023	0.0092	22	0.020	0.0081	29	0.76	ns
	rest	-0.013	0.0081	22	-0.010	0.0081	28	0.44	ns
	second	0.017	0.0047	22	0.018	0.0044	28	0.80	ns
409	first	0.023	0.0098	22	0.026	0.0066	28	0.17	ns
	rest	-0.006	0.0074	20	-0.009	0.0060	26	0.44	ns
	second	0.020	0.0093	21	0.016	0.0034	27	0.20	ns
410	first	0.032	0.0114	22	0.032	0.0092	28	0.76	ns
	rest	-0.020	0.0075	21	-0.016	0.0100	26	0.69	ns
	second	0.022	0.0058	21	0.022	0.0028	27	0.81	ns
400	first	0.036	0.0075	20	0.039	0.0070	28	0.66	ns
	rest	-0.015	0.0104	21	-0.022	0.0111	24	0.12	ns
	second	0.020	0.0052	21	0.021	0.0083	24	0.23	ns
404	first	0.028	0.0063	20	0.023	0.0063	28	0.02	*
	rest	0.000	0.0053	21	0.000	0.0071	24	0.37	ns
	second	0.016	0.0071	21	0.019	0.0070	24	0.28	ns

21. Median rates of body temperature change for each buffalo in group 2 over each work session in periods 1 and 2, with Mann-Whitney tests comparing period differences (Chapter 4).

Buffalo	Work session	Rates of body temperature change (°C/min) Period 1 (not infect.) Median	s.i.r.	n	Period 2 (infected) Median	s.i.r.	n	MW test P_{period}	Signif
406	first	0.026	0.0075	23	0.029	0.0055	29	0.9	ns
	rest	-0.006	0.0069	22	-0.012	0.0141	28	0.1	ns
	second	0.024	0.0103	22	0.020	0.0070	28	0.1	ns
408	first	0.025	0.0070	23	0.025	0.0080	29	0.8	ns
	rest	0.000	0.0066	22	-0.007	0.0086	28	0.1	ns
	second	0.014	0.0074	22	0.020	0.0046	28	0.1	ns
403	first	0.032	0.0050	22	0.031	0.0118	29	0.9	ns
	rest	-0.007	0.0126	20	-0.005	0.0050	28	0.8	ns
	second	0.024	0.0054	22	0.020	0.0040	27	0.1	ns
411	first	0.037	0.0100	22	0.022	0.0065	29	0.0	**
	rest	-0.020	0.0045	20	-0.010	0.0053	28	0.0	**
	second	0.019	0.0090	22	0.018	0.0050	27	0.7	ns
401	first	0.044	0.0130	19	0.041	0.0113	26	0.4	ns
	rest	-0.004	0.0140	18	-0.012	0.0128	25	0.2	ns
	second	0.023	0.0073	18	0.017	0.0065	25	0.2	ns
407	first	0.030	0.0099	20	0.027	0.0025	25	0.3	ns
	rest	-0.004	0.0040	19	-0.008	0.0088	24	0.2	ns
	second	0.017	0.0045	19	0.016	0.0045	24	0.6	ns

22. Median buffalo body temperatures (°C) immediately after work during each period with results of Mann-Whitney tests to compare period differences (Indonesian study, Chapter 4)

Group 1

	Period 1 (infected)			Period 2 (treated)			MW test		
Buffalo	**Median**	**s.i.r.**	**n**	**Median**	**s.i.r.**	**n**	**W**	**P_{period}**	**Signif**
402	41.3	0.55	23	40.8	0.53	29	700	0.09	ns
405 [$]	41.2	0.60	22	41.1	0.38	29	585	0.81	ns
409	41.0	0.52	22	40.5	0.33	26	626	0.07	ns
410 [$]	41.3	0.71	22	41.2	0.64	28	536	0.63	ns
400	42.4	0.38	21	41.7	0.20	28	693	<0.001	***
404	41.8	0.75	21	40.8	0.35	28	714	<0.001	***

[$] Buffalo that did not develop patent parasitaemias

Group 2

	Period 1 (not infect)			Period 2 (infected)			MW test		
Buffalo	**Median**	**s.i.r.**	**n**	**Median**	**s.i.r.**	**n**	**W**	**P_{period}**	**Signif**
406	41.3	0.64	20	40.6	0.48	29	614	0.02	*
408	41.2	0.45	17	41.3	0.40	29	401	0.99	ns
403	41.0	0.55	22	41.6	0.52	29	585	0.81	ns
411	41.3	0.63	21	40.8	0.60	29	684	0.004	**
401	42.4	0.55	21	41.5	0.45	29	650	0.03	*
407	41.8	0.30	21	40.7	0.40	29	596	0.24	ns

23. Liveweight of each buffalo over the study (Indonesian study, Chapter 4).

	Group 1						Group 2					
Buffalo	**402**	**405**	**409**	**410**	**400**	**404**	**406**	**408**	**403**	**411**	**401**	**407**
Team	**White**		**Red**		**Yellow**		**Green**		**Stripes**		**Blue**	
Expt. day	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**
-38	298	296	311	322	326	345	250	268	306	314	350	384
-22	284	284	298	312	312	332	236	256	290	296	336	364
-15	274	276	296	306	302	320	234	258	288	293	324	360
-8	292	290	300	312	316	334	248	270	302	298	342	374
-1	296	282	296	314	312	332	244	262	296	288	336	358
6	298	284	298	316	308	340	248	266	304	294	344	374
13	308	296	306	324	314	346	250	276	304	302	3444	378
20	310	296	306	322	318	352	258	278	310	306	358	380
27	313	306	306	328	316	356	261	286	314	314	358	390
34	324	320	290	330	328	372	274	296	322	322	358	384
41	324	318	298	336	330	370	272	296	320	320	354	390
47	330	328	308	342	330	374	278	306	324	330	364	384
55	334	320	298	338	326	382	276	308	322	330	360	392
62	334	326	310	344	324	380	272	312	326	338	360	392
69	346	340	322	350	340	392	286	320	334	346	378	400
76	348	334	312	356	338	396	292	322	342	352	382	408
83	348	336	322	354	338	406	290	326	336	352	380	412
90	340	336	324	350	340	396	292	322	332	354	370	400

24. Fresh weights of concentrate & Elephant grass offered to each buffalo during each period with the animal liveweights used to calculate the rations (Indonesian study, Chapter 4).

Buffalo	Initial animal LW (day -38)	Period 1 Concentrate	Elephant grass	Animal LW (day 46)	Period 2 Concentrate	Elephant grass
Group 1	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**	**(kg)**
402	298	3.0	25.0	330	3.5	25.0
405	296	3.0	25.0	328	3.5	25.0
409	311	3.0	25.0	308	3.5	25.0
410	322	3.5	25.0	342	3.5	25.0
400	326	3.5	25.0	330	3.5	25.0
404	345	3.5	25.0	374	3.5	30.0
Group 2						
406	250	3.0	20.0	278	3.0	25.0
408	268	3.0	20.0	306	3.5	25.0
403	306	3.0	25.0	324	3.5	25.0
411	314	3.0	25.0	330	3.5	25.0
401	350	3.5	30.0	364	3.5	30.0
407	384	3.5	30.0	384	3.5	30.0

25. Mean gross energy and crude protein intakes per unit of metabolic LW for each buffalo during each period (Indonesian study, Chapter 4).

Buffalo	GE intake (MJ/kg$^{0.75}$)				CP intake (g/kg$^{0.75}$)			
	Period 1		Period 2		Period 1		Period 2	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Group 1								
402	1.65	0.038	1.98	0.030	10.36	0.251	14.59	0.240
405	1.74	0.036	2.01	0.031	10.88	0.198	14.77	0.225
409	1.54	0.057	1.96	0.033	9.53	0.332	14.56	0.230
410	1.71	0.035	1.86	0.034	11.09	0.198	13.82	0.249
400	1.65	0.044	1.98	0.030	10.21	0.386	14.59	0.223
404	1.64	0.033	1.97	0.035	10.56	0.184	14.32	0.251
n	33		40		33		40	
Group 2								
406	1.65	0.043	2.10	0.040	10.89	0.199	15.63	0.296
408	1.49	0.047	1.89	0.040	9.93	0.186	14.11	0.276
403	1.65	0.035	1.89	0.033	10.37	0.202	14.06	0.241
411	1.67	0.038	1.95	0.029	10.52	0.208	14.33	0.222
401	1.81	0.040	2.08	0.033	11.18	0.260	15.10	0.261
407	1.68	0.039	1.96	0.032	10.55	0.223	14.21	0.244
n	33		40		33		40	

26. Sheep antibody responses to ovalbumin (OD values) measured by ELISA (Sheep study, Chapter 5).

		Day of the experiment (ELISA OD values)																									Primary peak	Days to primary peak	Secondary peak	Days to secondary peak
Group	Sheep	11	32	53	60	64	67	71	74	78	81	85	88	92	95	99	102	106	109	113	116	120	123	127	130	150				
EL	307	0.0350	0.0737	0.0841	0.0760	0.1329	0.2566	0.6548	0.6400	0.7658	0.5017	0.5281	0.5112	0.4664	0.4523	0.7421	1.2955	1.4529	1.4266	1.4707	1.3650	1.4108	1.2617	1.2869	1.3386	1.1882	0.7658	78	1.4707	113
	312	0.0390	0.0737	0.0731	0.0682	0.1119	0.1634	0.2599	0.2375	0.3837	0.2155	0.2080	0.1928	0.1714	0.3166	0.3670	1.2292	1.2540	1.2703	1.2809	1.1573	1.1002	1.1596	1.1645	1.0641	0.8922	0.3837	78	1.2809	113
	317	0.0900	0.0736	0.0872	0.0829	0.2290	0.2571	0.4755	0.5255	0.3535	0.2524	0.3991	0.6323	0.4888	0.7659	0.5824	1.2691	1.4671	1.4010	1.2425	1.2130	1.2555	1.2195	1.2265	1.2306	0.8698	0.6323	88	1.4671	106
	318	0.0922	0.0815	0.0929	0.1013	0.1345	0.6043	0.8373	0.7101	0.6204	0.6339	0.8386	0.8980	0.9349	0.6559	0.9671	1.4312	1.5652	1.5318	1.4790	1.4570	1.2670	1.2792	1.2667	1.1973	0.9086	0.9349	92	1.5652	106
	322	0.0929	0.0922	0.0914	0.0925	0.1212	0.1478	0.3420	0.3572	0.2805	0.2554	0.3758	0.3298	0.3166	0.4006	0.3213	0.4693	1.1176	1.0261	0.8560	0.6480	0.6305	0.4778	0.4251	0.3233	0.2769	0.3758	85	1.1176	106
	323	0.0943	0.0936	0.0857	0.0836	0.1618	0.1530	0.3353	0.4182	0.2294	0.4573	0.6000	0.5068	0.5050	0.6141	0.5096	1.1886	1.4147	1.3667	1.2230	1.0825	1.1850	1.1960	1.0951	1.0059	0.6888	0.6000	85	1.4147	106
RH	300	0.0474	0.0773	0.0778	0.0688	0.1003	0.2417	0.3691	0.2998	0.4545	0.3794	0.2974	0.3602	0.3528	0.2003	0.7056	1.3925	1.4465	1.4260	1.5347	1.4058	1.4371	1.3315	1.3406	1.4245	1.1871	0.4545	78	1.5347	113
	305	0.0484	0.0815	0.0763	0.0721	0.1440	0.2913	0.3029	0.3204	0.3936	0.2613	0.2261	0.2147	0.2013	0.2459	0.4269	1.1154	1.1738	1.2413	1.3192	1.0450	1.1708	1.0844	1.0359	0.9685	0.7219	0.3936	78	1.3192	113
	309	0.0464	0.0846	0.0789	0.0804	0.1169	0.0989	0.1221	0.1223	0.1562	0.1409	0.1548	0.1534	0.1798	0.2758	0.6014	1.3713	1.4371	1.4582	1.4738	1.3829	1.4207	1.2467	1.3085	1.3224	1.1634	0.1798	92	1.4738	113
	314	0.0449	0.0752	0.0768	0.0738	0.1213	0.3426	0.6294	0.4937	0.6256	0.4619	0.4517	0.4340	0.3388	0.5155	0.8113	1.0654	1.1768	1.3174	1.2151	1.1216	1.1300	1.0933	1.1251	1.0222	0.9131	0.6294	71	1.3174	109
	319	0.0800	0.0872	0.0786	0.0814	0.1205	0.2401	0.5808	0.3881	0.3488	0.3837	0.3838	0.3147	0.1894	0.7153	0.6783	1.3129	1.5674	1.5194	1.4250	1.3930	1.3960	1.3111	1.2177	1.2339	1.1234	0.5808	71	1.5674	106
	321	0.1028	0.0900	0.0943	0.0991	0.4615	0.5929	0.9864	0.9051	0.5344	0.7562	0.9636	0.8422	0.6706	0.8848	0.6238	1.2948	1.4759	1.4508	1.3090	1.1835	1.1935	1.1863	1.1497	1.0040	0.8432	0.9864	71	1.4759	106
EH	301	0.0489	0.1007	0.0830	0.0942	0.1457	0.2285	0.3680	0.3394	0.4500	0.2923	0.2497	0.2636	0.2102	0.0772	0.4797	1.2812	1.2738	1.3156	1.3242	1.2055	1.1042	1.1605	1.1340	1.1087	0.9035	0.4500	78	1.3242	113
	302	0.0449	0.0815	0.0737	0.0671	0.1429	0.1789	0.1800	0.1820	0.2704	0.2326	0.2405	0.2222	0.2207	0.1534	0.4953	1.3223	1.3074	1.3652	1.4247	1.3059	1.4396	1.2608	1.3149	1.2827	1.1237	0.2704	78	1.4396	120
	303	0.0659	0.0830	0.0903	0.0787	0.1783	0.2511	0.3393	0.3241	0.4276	0.2678	0.2377	0.2222	0.2157	0.2621	0.3429	1.0347	1.2475	1.2823	1.2696	1.1350	1.0694	1.0487	1.0708	1.0039	0.7853	0.4276	78	1.2823	109
	306	0.0479	0.0773	0.0752	0.0710	0.0959	0.1678	0.2621	0.3119	0.4560	0.3262	0.3697	0.3602	0.3687	0.4692	1.0280	1.4678	1.4628	1.4526	1.4851	1.3834	1.3685	1.2917	1.2507	1.3923	1.2451	0.4560	78	1.4851	113
	311	0.0514	0.0830	0.0815	0.0848	0.1965	0.3068	0.5114	0.4493	0.7339	0.4980	0.4651	0.4410	0.4250	0.5513	1.0566	1.3069	1.4529	1.3400	1.4351	1.3308	1.4093	1.2431	1.2577	1.4035	1.1715	0.7339	78	1.4529	106
	313	0.0335	0.0794	0.0783	0.0721	0.1539	0.2235	0.2737	0.2607	0.3562	0.2247	0.2266	0.2237	0.1908	0.2674	0.6397	1.1292	1.4247	1.2805	1.2244	1.1250	1.3531	1.0544	0.9161	1.0404	0.7252	0.3562	78	1.4247	106
RL	304	0.0659	0.0830	0.0903	0.0787	0.1783	0.2511	0.3393	0.3241	0.4276	0.2678	0.2377	0.2222	0.2157	0.2621	0.3429	1.0347	1.2475	1.2823	1.2696	1.1350	1.0694	1.0487	1.0708	1.0039	0.7853	0.4276	78	1.2823	109
	308	0.0365	0.0778	0.0757	0.0749	0.0920	0.1116	0.1347	0.1308	0.1223	0.1409	0.1358	0.1539	0.1430	0.2289	0.3247	1.2168	1.2673	1.3382	1.2476	1.3163	1.1737	1.1026	1.1835	1.2993	1.0340	0.1539	88	1.3382	109
	315	0.1068	0.0830	0.0820	0.0904	0.1175	0.2831	0.3856	0.3278	0.4989	0.3225	0.2798	0.3124	0.2446	0.2888	0.3442	1.1896	1.1827	1.4157	1.3125	1.2835	1.2632	1.2780	1.1525	1.0444	0.9481	0.4989	78	1.4157	109
	316	0.1798	0.1021	0.0936	0.1146	0.3464	0.7693	0.7773	0.6460	0.6584	0.6163	0.8446	0.7705	0.6461	0.7536	0.6858	1.2878	1.5507	1.4226	1.3905	1.3555	1.2195	1.0157	1.0147	0.9347	0.6841	0.8446	85	1.5507	106
	320	0.0815	0.0993	0.0943	0.0888	0.1013	0.1050	0.1987	0.2529	0.1815	0.2780	0.2341	0.2353	0.1791	0.2664	0.3837	0.7336	1.2219	1.2857	1.1195	1.1110	0.9370	1.0429	0.9185	0.8991	0.4629	0.2780	81	1.2857	109
	310	0.0409	0.1178	0.1050	0.1094	0.3744	0.5147	1.1626	1.0651	0.8759	0.9565	1.1012	1.1478	0.9152	1.1224	0.8634	1.1790	1.3258	1.2312	0.9360	0.9790	0.8470	1.1076	0.9112	0.5985	0.5177	1.1626	71	1.3258	106
EL	means	0.0739	0.0814	0.0857	0.0841	0.1486	0.2637	0.4841	0.4814	0.4389	0.3860	0.4916	0.5118	0.4805	0.5342	0.5816	1.1472	1.3786	1.3370	1.2587	1.1538	1.1415	1.0990	1.0775	1.0266	0.8041	0.6154	84.3	1.3860	108.3
RH		0.0617	0.0826	0.0804	0.0793	0.1774	0.3013	0.4984	0.4216	0.4189	0.3972	0.4129	0.3866	0.3221	0.4729	0.6412	1.2587	1.3796	1.4022	1.3795	1.2553	1.2913	1.2089	1.1963	1.1626	0.9920	0.5374	76.8	1.4481	110.0
EH		0.0488	0.0842	0.0803	0.0780	0.1522	0.2261	0.3224	0.3112	0.4490	0.3069	0.2982	0.2888	0.2719	0.2968	0.6737	1.2570	1.3615	1.3394	1.3605	1.2476	1.2907	1.1766	1.1574	1.2053	0.9924	0.4490	78.0	1.4015	111.2
RL		0.0852	0.0938	0.0902	0.0928	0.2016	0.3391	0.4997	0.4578	0.4607	0.4303	0.4722	0.4737	0.3906	0.4871	0.4908	1.1069	1.2993	1.3293	1.2126	1.1967	1.0850	1.0993	1.0419	0.9633	0.7387	0.5609	80.2	1.3664	108.0
EL	medians	0.0911	0.0776	0.0865	0.0832	0.1337	0.2100	0.4088	0.4718	0.3686	0.3563	0.4636	0.5090	0.4776	0.5332	0.5460	1.2492	1.4338	1.3839	1.2617	1.1852	1.2203	1.2078	1.1955	1.1307	0.8810	0.6162	85.0	1.4409	106.0
RH		0.0479	0.0830	0.0782	0.0771	0.1209	0.2665	0.4750	0.3542	0.4241	0.3816	0.3406	0.3375	0.2700	0.3956	0.6511	1.3039	1.4418	1.4384	1.3721	1.2832	1.2948	1.2165	1.1837	1.1280	1.0183	0.5177	74.5	1.4748	111.0
EH		0.0484	0.0823	0.0799	0.0754	0.1498	0.2260	0.3065	0.3180	0.4388	0.2801	0.2451	0.2436	0.2182	0.2648	0.5675	1.2941	1.3661	1.3278	1.3744	1.2557	1.3608	1.2018	1.1923	1.1957	1.0136	0.4388	78.0	1.4321	111.0
RL		0.0737	0.0912	0.0920	0.0896	0.1479	0.2671	0.3625	0.3259	0.4632	0.3002	0.2588	0.2738	0.2302	0.2776	0.3640	1.1843	1.2574	1.3119	1.2586	1.2093	1.1216	1.0756	1.0427	0.9693	0.7347	0.4632	79.5	1.3320	109.0

27. Sheep antibody responses to *B.abortus* (OD values) measured by ELISA (Sheep study, Chapter 5).

		Day of the experiment																									Primary	Days to	Secondary	Days to
		11	32	53	60	64	67	71	74	78	81	85	88	92	95	99	102	106	109	113	116	120	123	127	130	150	peak	primary	peak	secondary
Group	Sheep	(ELISA OD value)																										peak		peak
EL	307	0.0724	0.0994	0.1042	0.0921	0.1345	0.2001	0.4990	0.6653	0.7822	0.5616	0.4929	0.6671	0.6508	0.5733	0.8683	1.2473	1.2349	1.2957	1.2844	1.2809	1.3287	1.1518	1.1904	1.1095	1.1870	0.7822	78	1.3287	120
	312	0.0734	0.0946	0.1037	0.0859	0.4091	0.3864	0.6090	0.6831	0.7481	0.5894	0.5089	0.5901	0.5932	0.5950	0.9234	1.4152	1.5048	1.4589	1.4830	1.4233	1.4847	1.3780	1.4380	1.3450	1.3640	0.7481	78	1.5048	106
	317	0.1390	0.1406	0.1599	0.1721	0.5449	0.4816	0.5696	0.6890	0.5892	0.7527	0.5628	0.7273	0.8954	1.3311	1.1499	1.4456	1.5064	1.4950	1.3010	1.2856	1.3347	1.3448	1.3582	1.3487	1.0857	0.8954	92	1.5064	106
	318	0.1358	0.1293	0.1524	0.1487	0.3737	1.0002	1.0557	0.9717	0.9818	1.1951	0.9476	0.9895	0.9723	1.0352	1.2369	1.4343	1.4491	1.4619	1.2819	1.2865	1.1875	1.2856	1.2804	1.3234	1.0753	1.1951	81	1.4619	109
	322	0.1097	0.1084	0.1240	0.1014	0.1960	0.2258	0.1981	0.2293	0.1934	0.2990	0.2908	0.2535	0.2544	0.4901	0.6883	1.0750	1.4282	1.3903	1.1716	1.1183	1.0085	1.1603	1.1290	0.9028	0.7948	0.2990	81	1.4282	106
	323	0.0955	0.1073	0.1009	0.0950	0.4215	0.6960	1.1757	1.1694	1.0262	1.1715	0.9918	0.9909	0.8819	1.3188	1.3100	1.3978	1.4855	1.4836	1.2482	1.2622	1.2594	1.3478	1.3165	1.3526	1.0520	1.1757	71	1.4855	106
RH	300	0.0950	0.1047	0.1052	0.1320	0.2713	0.4367	0.7698	0.7363	0.8226	0.7527	0.5516	0.6911	0.6977	0.4514	0.8964	1.2879	1.3820	1.3209	1.4623	1.2516	1.3774	1.3278	1.3242	1.3010	1.0615	0.8226	78	1.4623	113
	305	0.0504	0.0782	0.0819	0.0787	0.3511	0.7679	1.0388	1.0195	1.0904	0.9370	0.7717	0.8211	0.6467	0.8923	1.2171	1.3404	1.3221	1.4173	1.4330	1.3696	1.4560	1.3776	1.2864	1.3430	1.3215	1.0904	78	1.4560	120
	309	0.0777	0.0883	0.0888	0.0798	0.1641	0.2230	0.2116	0.2221	0.3476	0.2552	0.2722	0.3795	0.4320	0.5505	0.7750	1.3587	1.3909	1.4578	1.4897	1.4467	1.4443	1.3691	1.4073	1.3580	1.2610	0.4320	92	1.4897	113
	314	0.0639	0.0893	0.0914	0.0742	0.1931	0.3025	0.5886	0.5860	0.8175	0.5637	0.8285	0.6962	0.7717	0.7721	0.8735	1.2072	1.3607	1.3524	1.3857	1.3482	1.3974	1.2824	1.4398	1.3080	1.1805	0.8285	85	1.4398	127
	319	0.0971	0.1062	0.1030	0.0950	0.1721	0.4152	0.8391	0.4465	0.6087	0.8111	0.7538	0.7631	0.8186	1.1550	0.8916	1.3898	1.5031	1.5282	1.3828	1.3192	1.2977	1.3413	1.2856	1.3247	1.2235	0.8391	71	1.5282	109
	321	0.1019	0.0858	0.0907	0.0886	0.2928	0.6247	1.0399	1.0193	0.7086	0.9834	0.8693	0.8162	0.6625	1.1427	1.1077	1.4863	1.4595	1.4718	1.2464	1.1534	1.1501	1.2673	1.2369	1.1077	0.8768	1.0399	71	1.4863	102
EH	301	0.0694	0.0909	0.0898	0.1122	0.2573	0.3858	0.5804	0.6208	0.7639	0.5421	0.4182	0.5539	0.5600	0.1283	0.6466	1.0220	1.1879	1.2052	1.1813	1.1131	1.1014	1.1807	1.0469	1.0255	0.8625	0.7639	78	1.2052	109
	302	0.0646	0.0845	0.0605	0.0764	0.2143	0.3362	0.6160	0.6653	0.7771	0.7002	0.6015	0.7283	0.7712	0.4590	0.9339	1.3681	1.3300	1.3326	1.3605	1.2926	1.3994	1.3202	1.2793	1.3130	1.3030	0.7771	78	1.3994	120
	303	0.0516	0.0829	0.0962	0.0870	0.8863	0.7024	0.6872	0.7445	0.8839	0.7135	0.6898	0.8252	0.8691	0.9468	1.0747	1.3577	1.4647	1.4740	1.4533	1.3872	1.3545	1.3011	1.3309	1.3565	1.2545	0.8863	64	1.4740	109
	306	0.0450	0.0819	0.0824	0.0680	0.2048	0.2815	0.3985	0.4587	0.5004	0.3654	0.3508	0.5626	0.4340	0.4209	0.7053	1.2320	1.2557	1.2705	1.2034	1.0926	0.9931	1.1771	1.1558	1.1560	1.0260	0.5626	88	1.2705	109
	311	0.0842	0.0782	0.0814	0.0708	0.6012	0.7756	0.9123	0.8462	1.0304	0.8778	0.7708	0.8431	0.8415	0.9550	0.9451	1.3929	1.4310	1.4127	1.4375	1.3974	1.4550	1.3500	1.3300	1.4850	1.3540	1.0304	78	1.4850	130
	313	0.0911	0.0729	0.0742	0.0680	0.1652	0.2771	0.6032	0.6596	0.7961	0.6126	0.5884	0.7518	0.8614	0.9269	1.0565	1.3865	1.5459	1.4438	1.5091	1.4238	1.5360	1.3291	1.4300	1.3945	1.2700	0.8614	92	1.5459	106
RL	304	0.0522	0.0776	0.0755	0.0897	0.2908	0.2484	0.6198	0.5707	0.7860	0.5663	0.4536	0.5712	0.6472	0.5686	0.7527	1.3622	1.2909	1.3817	1.4375	1.3399	1.2726	1.2936	1.2318	1.1735	1.0560	0.7860	78	1.4375	113
	308	0.0676	0.0983	0.0989	0.0814	0.2087	0.2414	0.3254	0.4020	0.3855	0.4602	0.4245	0.6799	0.7008	0.7399	0.9404	1.3880	1.4122	1.4227	1.2997	1.4282	1.4150	1.2687	1.3700	1.4030	1.2810	0.7008	92	1.4282	116
	315	0.0842	0.0914	0.0983	0.0798	0.1473	0.2357	0.4824	0.5717	0.6407	0.4581	0.7485	0.5059	0.5325	0.5335	0.6308	1.1631	1.0938	1.2543	1.3033	1.2755	1.2526	1.3140	1.2047	1.2705	0.9900	0.7485	85	1.3140	123
	316	0.1406	0.1551	0.1777	0.2030	0.4641	0.9236	0.9099	0.9094	0.9409	1.1377	1.0245	1.0016	0.9858	1.3525	1.0959	1.5110	1.5940	1.5324	1.4982	1.4281	1.3398	1.3200	1.3817	1.2360	1.1120	1.1377	81	1.5940	106
	320	0.1138	0.1230	0.1347	0.1141	0.2466	0.3232	0.4423	0.6224	0.5659	0.8286	0.6220	0.7082	0.6560	1.1176	1.0176	1.2782	1.4637	1.4822	1.3220	1.3001	1.2202	1.3622	1.3256	1.2986	1.0916	0.8286	81	1.4822	109
	310	0.0842	0.1503	0.1052	0.1179	0.2471	0.5858	0.7191	0.7033	0.6314	0.9590	0.8316	0.9168	1.0431	1.3615	1.2927	1.4809	1.5282	1.4178	1.2651	1.2767	1.2389	1.2381	1.2023	1.0207	1.2651	1.0431	92	1.5282	106
EL	mean	0.1043	0.1133	0.1242	0.1159	0.3466	0.4984	0.6845	0.7346	0.7201	0.7616	0.6325	0.7031	0.7080	0.8906	1.0295	1.3359	1.4348	1.4309	1.2950	1.2761	1.2673	1.2780	1.2854	1.2303	1.0931	0.8492	80.2	1.4526	108.8
RH		0.0810	0.0921	0.0935	0.0914	0.2407	0.4617	0.7480	0.6716	0.7326	0.7172	0.6745	0.6945	0.6715	0.8273	0.9602	1.3451	1.4030	1.4247	1.4000	1.3148	1.3538	1.3276	1.3300	1.2904	1.1541	0.8421	79.2	1.4770	114.0
EH		0.0677	0.0819	0.0808	0.0804	0.3882	0.4598	0.6329	0.6658	0.7920	0.6353	0.5699	0.7108	0.7229	0.6395	0.8937	1.2932	1.3692	1.3565	1.3575	1.2844	1.3066	1.2764	1.2621	1.2884	1.1783	0.8136	79.7	1.3967	113.8
RL		0.0904	0.1160	0.1150	0.1143	0.2674	0.4264	0.5831	0.6299	0.6584	0.7350	0.6841	0.7306	0.7609	0.9456	0.9550	1.3639	1.3971	1.4152	1.3543	1.3414	1.2898	1.2994	1.2860	1.2337	1.1326	0.8741	84.8	1.4640	112.2
EL	median	0.1026	0.1079	0.1141	0.0982	0.3914	0.4340	0.5893	0.6861	0.7651	0.6711	0.5359	0.6972	0.7664	0.8151	1.0366	1.4065	1.4673	1.4604	1.2831	1.2832	1.2941	1.3152	1.2984	1.3342	1.0805	0.8388	79.5	1.4737	106.0
RH		0.0864	0.0888	0.0911	0.0842	0.2322	0.4259	0.8045	0.6612	0.7631	0.7819	0.7628	0.7297	0.6801	0.8322	0.8940	1.3496	1.3865	1.4375	1.4094	1.3337	1.3874	1.3345	1.3053	1.3164	1.2020	0.8338	78.0	1.4743	113.0
EH		0.0670	0.0824	0.0819	0.0736	0.2358	0.3610	0.6096	0.6624	0.7866	0.6564	0.5950	0.7400	0.8063	0.6929	0.9395	1.3629	1.3805	1.3727	1.3990	1.3399	1.3770	1.3107	1.3047	1.3348	1.2623	0.8193	78.0	1.4367	109.0
RL		0.0842	0.1107	0.1020	0.1019	0.2468	0.2858	0.5511	0.5971	0.6361	0.6974	0.6852	0.6940	0.6784	0.9287	0.9790	1.3751	1.4380	1.4202	1.3127	1.3200	1.2626	1.3038	1.2787	1.2533	1.1018	0.8073	83.0	1.4599	111.0

28. Bull ages at the start of the experiment determined from their dentition, using the technique developed by Miller & Robertson (1945).

(Gambia study, Chapter 6).

Animal No.	No. of adult teeth visible	Estimated age (years)	Actual age if known
1	2	2 +	2 years 1 month
2	4	2.5 +	
3	4	2.5 +	
4	4	2.5 +	
5	3	2.5	
6	2	2 +	2 years 5 months
7	6	3 +	
8	2	2 +	
9	6	3 +	
10	6	3 +	
11	2 + 1 emerging	2 +	
12	6	3 +	
13	4 + 2 emerging	2.5 +	
14	6	3	
15	4	2.5 +	
16	6	3 +	
17	3	2.5	
18	6	3 +	
19	6	3 +	
20	6	3 +	
21	4	2.5 +	
22	4	2.5 +	2 years 1 month
23	6	3 +	
24	4	2.5 +	
25	5	2.5 +	
26	6	3 +	
27	5 + 1 emerging	3 +	
28	6	3 +	
29	6	3 +	
30	6	3 +	
31	8	4.5 +	
32	5	3 +	
33	8	4.5 +	
34	8	4.5 +	

29. An example of the calibration technique for correcting inter-plate variation in ELISA results.

Plate Ref:	20IgG1	Dilutions:	
Test date:	19/07/94	Antigen coating:	1:1600
Samples tested:	23/03/94	Serum dil:	1:25

PLATE LAYOUT

+ve control dilutions		1	2	3	4	5
1:3.125	A	+ve	+ve	+ve	1	
1:6.25	B	+ve	+ve	+ve	1	
1:12.5	C	+ve	+ve	+ve	2	
1:25	D	+ve	+ve	+ve	2	Animal
1:50	E	+ve	+ve	+ve	3	sera
1:150	F	+ve	+ve	+ve	3	
1:450	G	+ve	+ve	+ve	4	
1:1350	H	+ve	+ve	+ve	4	

OD READINGS

	1	2	3	4	5
A	0.402	0.442	0.427	0.138	
B	~~0.287~~	0.406	0.411	0.131	
C	0.364	0.378	0.352	0.221	
D	0.322	0.321	0.330	0.188	
E	0.288	0.300	0.289	0.185	
F	0.249	0.235	0.232	0.196	
G	0.186	0.181	0.197	0.188	
H	0.155	0.156	0.159	0.186	

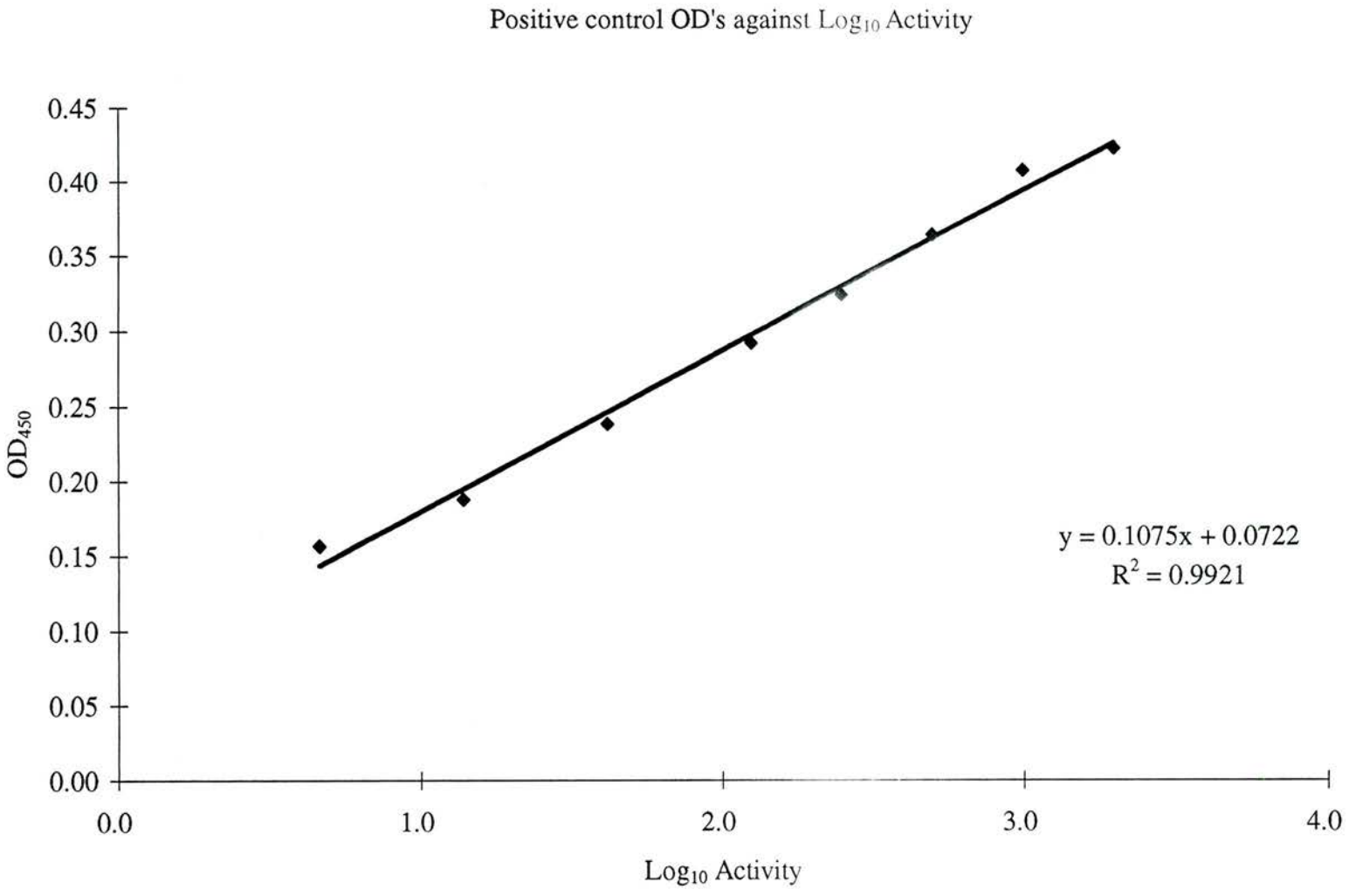
NB: The reading in B1 was deleted as differing from B2 and B3 by more than 10% of the anti-log of B2.

Dilution	Activity	Log_{10} Act.	Mean OD (cols. 1-3)
1:3.125	2000	3.3010	0.4237
1:6.25	1000	3.0000	0.4085
1:12.5	500	2.6990	0.3647
1:25	250	2.3979	0.3243
1:50	125	2.0969	0.2923
1:150	41.6667	1.6198	0.2387
1:450	13.8889	1.1427	0.1880
1:1350	4.6296	0.6655	0.1567

Animal	Mean OD (col. 4)
1	0.1345
2	0.2045
3	0.1905
4	0.1870

The log_{10} activity value for each serum OD is determined from the calibration line line overleaf (OD = 0.1075Log_{10} Activity + 0.0722). Corrected activity is the anti-log multiplied by the appropriate dilution factor. In this example the sera were diluted 1:25 and the control serum initial dilution was 3.125 (25/3.125 = 8) giving a dilution factor of 8.

Animal	Serum OD	Log_{10} Act	Corr Act.
1	0.1345	0.5795	30.4
2	0.2045	1.2307	68.0
3	0.1905	1.1005	50.4
4	0.1870	1.0679	46.8


Positive control OD's against Log10 Activity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
OD450
0.0
1.0
2.0
3.0
4.0
Log10 Activity
y = 0.1075x + 0.0722
R2 = 0.9921

30. Liveweight of each bull (kg) over the study (Gambian study, Chapter 6).

Group	Animal no.	Week 0	1	2	3	4	5	6	7	8	9	10	11	12
BN	1			172	174	175	175	173	170	165	161	161	161	161
	6	170	168	171	169	167	169	169	166	161	160	159	158	160
	7	178	178	181	180	177	178	179	179	179	176	175	175	176
	8	174	177	179	175	171	178	178	173	177	169	169	168	170
	13	204	200	204	209	207	205	208	206	205	204	203	204	205
	14	195	191	196	196	193	195	191	191	190	188	191	194	194
	15	197	191	196	196	191	191	189	186	186	185	184	186	187
	16	212	205	210	214	212	207	211	211	212	206	205	205	207
BW	2	178	170	171	173	168	171	167	162	160	161	162	166	165
	3	187	185	186	190	184	185	183	177	177	175	177	178	182
	4	186	180	188	187	184	180	179	173	171	165	161	156	155
	5	179	176	172	174	171	172	168	160	160	158	154	153	150
	9	202	195	202	202	198	197	195	192	188	187	189	192	193
	10	195	186	194	192	190	190	189	188	183	182	179	179	177
	11	197	188	192	190	188	186	187	188	186	183	181	177	173
	12	211	203	213	211	207	210	208	205	201	198	193	191	189
HN	21	176	175	178	180	181	183	187	184	186	185	183	189	189
	22	175	178	182	185	180	185	187	179	173	173	171	174	175
	23	175	179	181	183	179	182	180	177	175	170	173	170	167
	24	185	183	185	182	184	185	184	183	181	181	177	179	181
	25	196	188	194	201	199	201	200	195	191	190	189	188	188
	29	202	205	208	209	209	211	210	206	206	200	194	197	199
	31	198	196	199	201	200	201	201	197	200	197	197	199	200
	32	202	206	209	211	209	210	206	205	203	206	203	205	203
HW	17	172	164	173	174	172	175	175	176	176	175	176	178	179
	18	176	166	177	179	176	180	178	174	173	168	167	169	169
	19	184	179	183	186	183	187	187	184	182	183	178	177	175
	20	182	176	180	172	173	178	177	177	176	175	173	173	173
	26	201	194	200	203	200	203	204	202	200	198	192	187	189
	27	198	194	195	197	191	197	199	195	191	188	185	186	184
	28	205	196	204	206	204	207	205	196	197	191	190	189	191
	30	206	204	205	208	205	206	205	201	199	199	196	195	197
Control	33	184	186	191	190	187	190	190	189	191	190	194	196	199
	34	203	196	208	204	204	205	207	208	213	217	215	220	224
Mean	BN	190.0	187.1	188.4	188.8	186.6	187.1	187.0	185.1	184.3	180.9	180.7	181.2	182.3
	BW	191.9	185.4	189.5	189.6	186.3	186.1	184.3	180.4	178.1	176.0	174.3	173.9	172.8
	HN	188.6	188.8	191.9	193.7	192.6	194.3	193.9	190.6	189.1	187.5	185.8	187.3	187.4
	HW	190.5	184.1	189.6	190.4	188.0	191.3	190.9	187.9	186.4	184.3	181.9	181.6	181.8
	Control	193.5	191.0	199.3	197.0	195.5	197.5	198.3	198.3	201.8	203.5	204.3	207.8	211.3
s.e.	BN	6.08	5.04	5.26	5.99	5.94	5.06	5.50	5.83	6.30	6.27	6.32	6.57	6.58
	BW	4.06	3.69	4.98	4.56	4.55	4.59	4.80	5.45	5.03	4.91	4.96	5.21	5.48
	HN	4.32	4.32	4.39	4.56	4.60	4.39	3.93	4.02	4.49	4.60	4.19	4.44	4.49
	HW	4.79	5.27	4.48	5.21	4.91	4.69	4.66	4.11	3.98	3.99	3.62	3.15	3.47
	Control	9.50	5.00	8.75	7.00	8.50	7.50	8.25	9.75	10.75	13.50	10.25	12.25	12.25

31. Weekly mean lap times (mm:ss) for each team and each group over the study.

(Gambian study, Chapter 6).

Week:		1	2	3	4	5	6	7	8	9	10	11	12
Status		Healthy				Pre-patent		Parasitaemic					
Load (as % LW)		17.0	10.0	10.0	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Team	Group	Mean lap times (mm:ss)											
1	HW	30:41	27:51	25:48	25:47	24:20	26:38	35:42	48:22	43:36			
2	HW	47:36	31:14	29:27	28:51	26:40	33:31	41:21	40:58				
3	HW	50:32	30:41	27:45	31:30	25:19	31:57	38:55	36:57	32:06	34:29	35:04	
4	HW	34:22	26:37	24:18	25:17	27:32	27:23	28:34	36:48	33:32	29:12	29:59	29:40
5	BW	38:04	29:13	26:37	26:46	29:06	36:16	44:15	52:06				
6	BW	35:52	28:10	27:27	27:05	25:44	27:39	41:07					
7	BW	38:00	29:21	26:19	27:37	26:44	27:34	32:49	34:05	32:02	30:47	30:11	29;38
8	BW	36:15	28:03	24:37	25:40	25:15	27:57	31:23	32:26	33:27	30:28	31:31	31:58
9	Control	34:45	28:32	27:18	26:53	26:00	26:29	26:55	28:00	26:23	26:12	25:09	24:50
Group mean HW		40:12	29:06	26:47	27:51	25:58	29:52	36:08	40:28	36:17	31:51	32:31	29:40
Group mean BW		37:03	28:42	26:15	26:47	26:42	29:52	37:24	36:12	32:45	30:38	30:51	30:48
Team	Group	Standard errors of the means											
1	HW	01:02	00:21	00:34	00:31	00:21	00:34	01:21	02:58	01:27			
2	HW	03:54	00:48	01:53	00:19	00:26	00:47	01:25	01:55				
3	HW	07:41	00:42	01:08	02:04	00:18	03:51	02:26	00:35	00:22	01:11	01:53	
4	HW	01:41	00:28	00:51	00:26	01:03	00:27	00:36	01:50	00:51	00:32	00:28	00:55
5	BW	02:12	00:56	00:38	00:23	01:25	01:51	01:32	03:05				
6	BW	02:05	00:25	00:48	00:29	00:13	00:22	03:25					
7	BW	02:36	01:09	00:43	00:17	00:44	00:28	00:53	00:50	00:50	00:31	00:27	00:23
8	BW	01:19	00:33	00:25	00:23	00:17	00:21	00:33	00:42	00:47	00:54	00:21	00:30
9	Control	02:00	00:32	00:23	00:21	00:17	00:19	00:38	00:22	00:23	00:29	00:32	00:24
Group mean HW		02:14	00:22	00:37	00:37	00:20	01:02	00:57	01:11	00:52	00:48	01:02	00:55
Group mean BW		01:02	00:24	00:21	00:12	00:26	00:38	01:08	01:39	00:35	00:31	00:15	00:22
Team		Sample size (n)											
1		16	19	20	20	20	20	20	14	19			
2		15	19	19	20	20	20	20	8				
3		13	19	20	20	20	20	20	16	20	16	20	
4		16	19	20	20	20	20	20	16	20	16	20	20
5		15	19	20	20	20	20	20	3				
6		15	19	20	20	20	20	20					
7		16	19	20	20	20	20	20	10	20	16	20	20
8		16	19	20	20	20	20	20	7	20	16	20	20
9		8	17	16	20	13	16	16	16	20	16	20	20
Group mean HW		60	76	79	80	80	80	80	54	59	32	40	20
Group mean BW		62	76	80	80	80	80	80	20	40	32	40	40

32. Weekly mean speeds (ms^{-1}) for each team and each group over the study (Gambian study, Chapter 6).

Week		1	2	3	4	5	6	7	8	9	10	11	12
Status:		Healthy				Pre-patent		Parasitaemic					
Load (as % LW)		17.0	10.0	10.0	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Team	Group	Mean speeds (ms^{-1})											
1	HW	1.14	1.23	1.34	1.34	1.41	1.30	0.99	0.74	0.80			
2	HW	0.80	1.11	1.23	1.19	1.29	1.03	0.85	0.85				
3	HW	0.85	1.13/	1.27	1.15	1.36	1.21	0.94	0.93	1.07	1.01	1.02	
4	HW	1.03	1.29	1.43	1.36	1.27	1.26	1.21	0.96	1.03	1.18	1.15	1.17
5	BW	0.93	1.19	1.30	1.28	1.22	0.99	0.79	0.66				
6	BW	0.99	1.22	1.27	1.27	1.33	1.24	0.92					
7	BW	0.95	1.19	1.32	1.24	1.30	1.25	1.06	1.01	1.08	1.12	1.14	1.16
8	BW	0.97	1.23	1.40	1.34	1.36	1.23	1.10	1.06	1.04	1.14	1.09	1.08
9	Control	1.01	1.21	1.26	1.28	1.32	1.30	1.28	1.23	1.30	1.31	1.38	1.39
Group mean HW		0.96	1.19	1.32	1.26	1.33	1.20	0.99	0.88	0.97	1.09	1.08	1.17
Group mean BW		0.96	1.21	1.32	1.29	1.30	1.18	0.97	0.98	1.06	1.13	1.11	1.12
Team	Group	Standard errors of the means											
1	HW	0.040	0.017	0.027	0.024	0.020	0.026	0.037	0.039	0.021			
2	HW	0.077	0.027	0.059	0.015	0.019	0.024	0.028	0.037				
3	HW	0.099	0.023	0.042	0.050	0.016	0.063	0.045	0.014	0.012	0.033	0.040	
4	HW	0.053	0.023	0.037	0.024	0.037	0.018	0.024	0.033	0.026	0.021	0.018	0.032
5	BW	0.042	0.031	0.023	0.018	0.043	0.047	0.027	0.040				
6	BW	0.040	0.017	0.030	0.022	0.012	0.016	0.065					
7	BW	0.050	0.034	0.027	0.013	0.029	0.020	0.028	0.023	0.028	0.018	0.017	0.015
8	BW	0.042	0.024	0.023	0.020	0.011	0.016	0.020	0.023	0.024	0.032	0.012	0.017
9	Control	0.054	0.022	0.017	0.017	0.015	0.016	0.030	0.016	0.020	0.025	0.032	0.021
Group mean HW		0.038	0.014	0.023	0.018	0.014	0.021	0.023	0.020	0.019	0.025	0.024	0.032
Group mean BW		0.022	0.014	0.014	0.010	0.015	0.018	0.023	0.034	0.018	0.018	0.011	0.013
Team		Sample size (n)											
1		16	19	20	20	20	20	20	14	19			
2		15	19	19	20	20	20	20	8				
3		13	19	20	20	20	20	20	16	20	16	20	
4		16	19	20	20	20	20	20	16	20	16	20	20
5		15	19	20	20	20	20	20	3				
6		15	19	20	20	20	20	20					
7		16	19	20	20	20	20	20	10	20	16	20	20
8		16	19	20	20	20	20	20	7	20	16	20	20
9		8	17	16	20	13	16	16	16	20	16	20	20
Group mean HW		60	76	79	80	80	80	80	54	59	32	40	20
Group mean BW		62	76	80	80	80	80	80	20	40	32	40	40

33. Mean daily rice bran intake for each bull during each week of the study as a percentage of bran offered, (n = 7),(Gambian study, Chapter 6).

NB: At the start of week 8, sesame cake was introduced and when necessary the bran - sesame mixture was hand fed to ensure 100% intake.

	Week	1		2		3		4		5		6		7		8		9		10		11		12	
Group	Animal	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
BN	1	100	0.0	100	0.0	100	0.0	100	0.0	62	17.9	72	17.9	87	13.1	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	6	100	0.0	100	0.0	100	0.0	100	0.0	87	13.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	7	100	0.0	100	0.0	2	0.8	2	0.4	3	0.3	3	0.6	32	17.7	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	8	100	0.0	100	0.0	2	1.1	1	0.2	12	6.0	2	0.5	31	17.9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	13	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	14	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	82	12.3	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	15	100	0.0	100	0.0	61	15.2	86	14.1	55	16.8	69	16.5	41	16.5	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	16	100	0.0	100	0.0	100	0.0	88	11.5	11	3.8	26	13.6	32	17.7	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
BW	2	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	3	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	4	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	5	100	0.0	100	0.0	91	9.0	100	0.0	100	0.0	100	0.0	73	17.5	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	90	10.3	55	18.4	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	10	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	11	100	0.0	100	0.0	34	17.1	3	0.8	2	0.2	19	10.5	61	15.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	12	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	87	13.3	45	19.3	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
HN	21	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	22	100	0.0	100	0.0	100	0.0	100	0.0	87	12.7	46	19.0	93	7.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	23	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	39	16.5	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	24	100	0.0	100	0.0	87	12.7	53	18.3	10	7.1	37	13.8	40	16.7	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	25	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	86	13.9	55	16.9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	29	100	0.0	100	0.0	100	0.0	100	0.0	87	13.5	100	0.0	66	16.4	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	31	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	86	13.9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	32	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	87	13.5	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
HW	17	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	18	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	19	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	20	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	26	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	27	100	0.0	100	0.0	100	0.0	100	0.0	88	11.5	62	17.9	30	18.1	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	28	100	0.0	100	0.0	32	17.7	28	11.3	5	1.9	9	4.7	31	17.9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	30	100	0.0	100	0.0	100	0.0	86	14.2	86	13.9	26	11.2	30	18.1	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
Control	33	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	34	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0

34. Mean daily groundnut hay intake for each animal during each week of the study as a percentage of the hay offered (n = 7), (Gambian study, Chapter 6).

	Week	1		2		3		4		5		6		7		8		9		10		11		12	
Group	Animal	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
BN	1	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.8	97	1.7	100	0.0	100	0.0	100	0.0	100	0.0
	6	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.8	100	0.2	92	4.3	100	0.0	100	0.0	100	0.0	100	0.0
	7	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	8	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	1.2	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	13	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.7	98	1.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	14	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.5	98	1.1	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	15	100	0.0	100	0.0	100	0.0	99	0.5	100	0.0	98	1.3	96	2.8	99	0.5	98	0.6	100	0.0	100	0.5	100	0.0
	16	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
BW	2	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	3	100	0.0	100	0.0	100	0.0	98	1.5	100	0.0	95	2.5	86	4.0	95	2.0	98	1.1	98	1.1	100	0.0	100	0.0
	4	100	0.0	100	0.0	100	0.0	100	0.3	100	0.0	96	1.0	92	4.3	96	3.0	100	0.0	100	0.0	100	0.0	100	0.0
	5	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.6	99	0.6	100	0.0	100	0.0	98	1.6	99	0.8
	9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.8	97	1.3	100	0.2	100	0.0	100	0.0	100	0.0	100	0.0
	10	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	98	1.1	99	0.9	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	11	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	98	1.5	98	1.3	100	0.2	100	0.0	100	0.0	100	0.0	100	0.0
	12	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.9	99	0.5	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0
HN	21	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	97	1.8	97	0.8	95	1.8	99	0.7	96	2.5	97	2.1	97	1.5
	22	100	0.0	100	0.0	100	0.0	99	0.8	100	0.0	91	2.2	37	11.3	10	2.2	44	7.0	77	3.2	84	2.3	88	4.0
	23	100	0.0	100	0.0	100	0.0	99	1.4	98	1.6	97	1.4	84	4.0	77	4.8	80	2.8	85	2.8	74	1.6	81	6.0
	24	100	0.0	100	0.0	100	0.0	99	0.9	98	1.1	94	1.4	92	2.7	75	1.8	85	1.7	86	0.8	85	3.1	87	1.9
	25	100	0.0	100	0.0	100	0.0	99	0.8	92	5.7	87	3.1	76	2.4	71	2.3	82	2.2	77	4.5	72	3.0	80	3.9
	29	100	0.0	100	0.0	100	0.0	99	0.5	98	1.7	96	1.3	89	2.4	81	2.6	88	3.2	90	2.0	86	2.4	91	2.6
	31	100	0.0	100	0.0	100	0.0	100	0.0	100	0.3	91	3.2	84	3.4	79	4.4	89	2.0	88	3.7	89	3.0	93	1.9
	32	100	0.0	100	0.0	100	0.0	99	1.2	98	2.3	95	1.1	89	2.7	84	3.2	86	3.3	90	2.0	90	3.2	92	2.5
HW	17	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	98	0.8	95	1.6	94	2.2	97	0.8	100	0.0	100	0.0	99	0.6
	18	100	0.0	100	0.0	100	0.0	99	1.1	100	0.0	97	1.3	87	3.3	75	2.5	81	2.9	95	1.3	95	2.0	99	1.0
	19	100	0.0	100	0.0	100	0.0	99	0.6	98	1.8	97	1.0	92	2.1	91	2.4	97	0.9	93	1.5	81	2.9	94	2.3
	20	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0	99	0.3	98	0.8	96	2.0	100	0.0	99	1.4	96	2.7	92	2.0
	26	100	0.0	100	0.0	100	0.0	99	1.1	100	0.0	99	0.6	97	0.9	92	1.8	98	0.8	99	0.7	96	2.0	99	0.6
	27	100	0.0	100	0.0	100	0.0	100	0.4	99	1.1	96	1.7	92	3.6	85	2.3	85	3.3	94	2.8	95	1.9	97	1.7
	28	100	0.0	100	0.0	98	1.5	100	0.0	100	0.0	99	0.5	94	2.1	90	2.8	96	2.6	99	1.0	100	0.5	100	0.0
	30	100	0.0	100	0.0	100	0.0	100	0.2	100	0.0	98	0.6	90	4.0	95	2.9	92	1.8	95	1.5	97	1.8	99	0.5
Control	33	100	0.0	100	0.0	100	0.0	99	0.8	100	0.0	100	0.3	100	0.0	98	2.5	100	0.0	100	0.0	100	0.0	100	0.0
	34	100	0.0	100	0.0	100	0.0	99	0.5	100	0.0	99	0.3	100	0.2	100	0.0	100	0.0	100	0.0	100	0.0	100	0.0

NB: Anomalies in the s.e. figures whereby a mean plus its' s.e. equal more than 100% are due to rounding up of numbers; e.g. HN23 week 4 is actually 98.6 +/- 1.4%.

35. Mean daily andropogon grass intake per unit of metabolic liveweight (g DM /kg$^{0.75}$ /day), for each bull during each week of the study (n= 7), (Gambian study, Chapter 6).

	Week	1		2		3		4		5		6		7		8		9		10		11		12	
Group	Animal	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
BN	1			31.2	1.92	32.5	1.05	26.3	1.40	23.1	0.68	15.5	1.13	14.9	1.68	12.8	0.76	10.2	0.44	14.5	0.34	15.4	1.49	14.5	2.52
	6	24.5	2.51	28.4	0.50	24.0	1.08	24.0	1.01	27.1	2.44	15.3	1.84	14.8	1.81	11.5	0.74	14.3	0.59	12.4	0.53	15.0	2.10	8.7	1.10
	7	31.7	1.99	31.7	1.74	31.9	0.65	20.8	1.62	14.9	1.07	12.2	1.40	13.9	1.55	13.3	1.07	10.5	0.88	11.2	1.32	10.7	1.11	9.9	1.32
	8	32.7	2.78	38.8	1.00	22.5	2.34	21.1	4.25	30.6	0.58	22.8	1.06	20.3	1.09	17.1	0.63	12.8	1.16	18.9	1.32	18.5	1.26	16.2	0.82
	13	26.2	2.26	29.9	5.03	40.0	2.44	30.6	0.99	31.1	1.54	21.1	0.40	21.9	0.99	18.2	0.68	19.0	0.69	19.0	0.99	16.3	1.00	12.9	0.58
	14	38.5	1.98	36.8	1.39	30.7	0.55	31.3	2.14	27.8	0.70	20.9	1.01	14.7	2.31	9.4	1.33	12.9	0.71	18.3	1.22	11.0	0.39	15.2	1.12
	15	20.8	1.12	21.5	1.82	25.8	1.62	22.7	0.46	21.1	1.96	11.7	1.53	15.3	1.38	10.9	1.17	8.8	0.67	10.2	0.58	10.5	1.10	9.9	1.38
	16	35.2	1.88	29.9	1.12	32.3	2.41	30.4	1.26	33.1	1.67	26.6	0.44	23.1	2.96	19.3	0.75	15.0	0.44	18.1	0.99	20.9	0.68	17.7	1.26
BW	2	29.6	0.84	41.0	1.49	38.6	0.55	36.7	2.65	32.5	1.63	27.3	0.75	21.9	0.92	22.0	0.59	26.4	1.99	26.6	1.26	23.6	2.67	17.4	1.89
	3	41.4	3.26	45.7	1.48	42.6	0.90	42.8	0.74	38.9	2.31	26.4	1.04	30.5	2.95	25.1	0.24	31.2	1.57	32.1	0.93	35.3	1.72	38.1	1.17
	4	33.3	1.47	36.3	1.42	31.7	1.52	29.0	0.88	27.0	0.65	20.5	0.88	16.2	2.89	9.4	0.63	11.8	0.67	10.0	0.65	6.6	0.57	8.9	0.44
	5	27.6	0.97	33.6	0.93	33.1	1.42	27.4	2.11	27.5	1.99	15.2	1.21	13.8	2.32	10.9	1.34	12.0	1.01	10.3	0.75	5.2	1.42	4.0	0.46
	9	42.2	1.84	40.9	3.92	46.0	0.60	42.9	0.44	38.1	0.79	33.8	0.89	26.8	2.17	29.1	1.20	31.4	1.10	35.7	1.24	34.5	2.24	35.1	1.63
	10	39.7	2.20	36.5	3.95	37.1	2.96	35.7	1.80	33.8	0.45	29.7	1.26	19.0	2.00	21.4	1.35	31.8	1.09	27.7	1.21	19.8	1.14	26.4	0.90
	11	14.9	1.65	18.4	2.25	22.7	0.74	21.9	2.08	20.8	0.69	15.3	1.04	16.9	1.46	13.5	0.63	15.1	1.43	19.5	1.57	13.4	0.36	10.5	0.87
	12	34.9	1.63	45.1	2.95	38.5	1.37	34.3	0.96	33.2	1.36	22.8	0.71	22.2	1.79	16.1	1.39	11.2	0.87	17.4	1.34	14.7	0.62	14.9	1.05
HN	21	9.6	0.89	9.4	0.40	10.2	0.67	7.5	0.86	11.7	1.75	6.6	0.68	8.2	0.70	4.3	0.44	3.1	0.31	2.7	0.60	2.7	0.62	5.2	1.26
	22	8.6	1.62	9.7	0.49	14.7	1.30	17.1	0.50	11.8	1.36	8.5	0.50	14.6	1.64	33.9	1.65	20.9	2.01	11.1	0.89	6.1	0.45	5.2	0.64
	23	8.6	0.42	10.9	0.51	10.9	0.42	10.5	0.29	10.3	0.27	4.7	0.57	3.1	0.70	1.9	0.25	1.7	0.20	1.5	0.07	1.3	0.22	1.7	0.14
	24	7.7	0.95	5.6	1.09	5.7	1.42	4.2	0.59	6.3	1.43	4.7	0.59	2.6	0.34	2.8	0.38	2.2	0.38	1.5	0.08	2.2	0.42	3.4	0.27
	25	10.6	0.96	9.9	0.51	11.5	1.10	7.4	0.37	3.2	0.87	8.0	0.38	4.6	0.62	2.6	0.47	2.8	0.23	3.6	0.12	2.6	0.48	3.8	0.11
	29	7.0	0.93	5.5	0.68	6.7	1.22	7.0	1.60	5.1	0.79	3.0	0.14	3.3	0.21	3.0	0.15	1.4	0.29	1.7	0.05	2.3	0.32	1.7	0.10
	31	9.9	0.70	14.3	0.95	14.9	0.68	12.0	0.63	8.1	1.06	5.2	0.13	4.5	0.22	4.3	0.32	3.0	0.23	3.1	0.16	3.0	0.18	3.0	0.15
	32	6.9	0.64	4.4	1.45	5.6	0.62	4.4	0.70	6.0	0.45	4.6	0.48	5.2	0.58	3.6	0.16	3.0	0.19	3.3	0.28	2.3	0.47	3.1	0.16
HW	17	9.7	0.68	10.4	0.57	8.8	0.92	10.7	0.09	13.7	1.40	9.1	0.32	7.3	0.94	5.7	1.66	2.3	0.58	1.6	0.25	2.4	0.58	8.8	2.34
	18	10.1	0.54	11.3	0.29	11.7	0.34	12.7	0.76	8.0	0.90	4.7	0.57	5.2	0.74	3.4	0.14	1.9	0.41	2.8	0.38	3.3	0.25	5.7	1.03
	19	9.1	0.64	9.9	0.59	8.2	1.03	11.8	0.40	11.3	1.24	5.2	0.85	7.2	0.80	4.9	0.29	4.1	0.30	3.8	0.27	3.7	0.16	3.5	0.19
	20	9.5	0.67	5.9	1.24	7.7	0.91	8.0	0.79	6.1	0.35	3.5	0.72	3.1	0.56	3.3	0.69	2.8	0.43	1.4	0.13	1.6	0.32	1.4	0.17
	26	7.8	0.68	7.2	1.14	5.3	0.76	6.1	0.36	3.9	0.37	4.8	1.12	11.0	1.00	7.7	0.25	4.6	0.59	4.0	0.13	4.5	0.51	5.2	1.07
	27	4.6	0.77	6.2	1.13	4.9	1.07	6.0	0.30	5.3	1.15	7.3	1.54	2.9	0.36	2.4	0.40	1.4	0.07	2.1	0.27	2.6	0.35	1.6	0.11
	28	8.0	0.21	13.8	1.68	15.4	1.07	15.3	1.55	8.1	0.12	5.6	0.66	6.2	0.62	2.9	0.56	3.3	0.32	3.2	0.14	2.6	0.46	5.0	0.18
	30	8.6	0.81	12.3	0.59	16.3	2.54	13.3	1.71	5.9	0.79	4.1	0.82	5.0	0.74	2.5	0.50	2.7	0.30	2.6	0.29	1.7	0.14	2.8	0.21
Control	33	9.5	0.65	16.2	1.24	17.7	0.96	13.9	0.46	11.0	0.74	11.1	0.27	11.2	0.46	12.4	0.71	13.1	1.13	15.1	1.37	16.2	1.46	19.1	0.95
	34	8.7	0.93	10.8	0.60	25.0	2.60	12.2	1.52	8.4	0.85	13.5	1.67	17.8	0.81	20.1	0.35	22.0	1.03	21.0	0.71	20.2	0.57	19.4	1.05

36. Estimated daily ME intake per unit of metabolic liveweight (MJ/kg$^{0.75}$/day) for each animal for each week of the study, (n = 7), with mean daily intakes pre-infection (weeks 1-4) and once parasitaemic (weeks 6-12), (Gambian study, Chapter 6).

Treatment	Animal	Week 1	2	3	4	5	6	7	8	9	10	11	12	Pre-infection		Parasitaemic	
														mean	s.e.	mean	s.e.
BN	1		637	653	596	566	507	505	598	595	636	633	623	629	16.9	585	21.4
	6	596	618	598	594	617	522	521	580	628	625	639	584	601	5.6	586	18.5
	7	632	626	590	505	470	435	460	582	563	580	567	556	588	29.2	535	22.9
	8	640	682	528	516	590	519	512	615	596	648	639	616	592	41.1	592	20.9
	13	598	618	695	620	637	544	546	621	625	634	604	575	633	21.2	593	14.2
	14	703	681	645	646	629	571	506	584	609	651	584	611	669	14.1	588	16.9
	15	573	569	590	577	562	487	491	601	578	606	593	587	577	4.5	563	19.5
	16	657	610	631	606	606	550	522	617	592	623	635	606	626	11.7	592	15.7
BW	2	630	712	700	686	661	618	582	689	715	722	683	635	682	18.1	663	19.9
	3	692	721	701	697	684	566	571	658	714	721	742	753	703	6.3	675	29.8
	4	640	648	624	601	604	535	490	556	597	601	580	599	628	10.5	565	15.6
	5	604	654	652	612	622	527	510	605	615	623	573	571	631	13.2	575	16.4
	9	724	703	750	724	701	654	576	733	749	784	760	761	725	9.4	717	28.2
	10	721	683	699	684	682	636	555	687	762	746	679	731	697	8.8	685	27.3
	11	534	553	562	535	539	488	515	623	636	682	636	622	546	6.9	600	26.8
	12	658	718	681	647	646	550	526	612	577	641	616	619	676	15.7	592	15.7
HN	21	782	769	785	749	794	704	716	766	779	777	750	767	771	8.2	751	11.3
	22	766	760	805	815	783	659	390	471	593	749	742	752	787	13.7	622	54.9
	23	763	772	782	768	776	712	587	670	693	730	652	706	771	4.1	679	18.2
	24	745	721	741	688	693	653	617	649	694	721	704	717	724	12.9	679	15.0
	25	827	803	812	766	704	684	570	659	731	717	670	729	802	12.9	680	21.3
	29	754	732	756	740	726	683	630	689	732	775	736	753	745	5.7	714	18.7
	31	799	820	836	803	790	693	635	704	758	761	755	773	815	8.4	726	19.0
	32	750	722	741	716	737	695	659	724	715	764	738	755	732	7.9	721	13.7
HW	17	818	791	795	799	833	763	719	806	794	816	800	839	801	6.1	791	14.8
	18	814	785	799	795	775	713	654	675	708	826	804	844	798	6.0	746	29.0
	19	766	760	754	771	767	696	675	760	782	776	696	776	763	3.7	737	17.5
	20	778	737	791	776	767	727	702	800	814	818	789	753	771	11.8	772	17.1
	26	789	765	761	753	756	731	765	814	826	858	847	863	767	7.8	815	18.8
	27	765	773	773	781	769	729	652	749	744	831	827	838	773	3.2	767	25.9
	28	785	805	783	783	729	693	688	772	826	862	847	859	789	5.3	792	28.7
	30	768	791	827	792	754	683	636	798	771	812	804	816	795	12.2	760	27.0
Control	33	825	859	888	849	844	822	819	909	925	942	930	937	855	13.0	898	20.4
	34	790	772	902	791	782	793	813	919	913	926	891	873	814	29.8	876	20.0

37. Median body temperature (degrees C) of each bull at 08.00 h and 12.00 h during each period of the study, together with the median temperature change between 08.00 and 12.00 h (Gambia study, Chapter 6).

		Uninfected									Pre-patent									Parasitaemic								
		08.00hrs			12.00hrs			Temp. change			08.00hrs			12.00hrs			Temp. change			08.00hrs			12.00hrs			Temp. change		
Group	Animal	median	s.i.r.	n	median	s.i.r.	n	median	s.i.r.	n	median	s.i.r.	n	median	s.i.r.	n	median	s.i.r.	n	median	s.i.r.	n	median	s.i.r.	n	median	s.i.r.	n
BN	1	36.86	0.29	20	37.99	0.07	5	1.03	0.30	5	36.79	0.31	5	38.13	0.41	5	1.34	0.36	5	37.61	0.50	34	39.74	0.58	33	1.80	0.50	33
	6	36.58	0.35	20	37.79	0.18	5	1.23	0.11	5	36.36	0.12	5	37.99	0.22	5	1.54	0.09	5	37.24	0.32	34	38.63	0.53	34	1.29	0.39	34
	7	36.37	0.33	20	38.06	0.09	5	1.78	0.25	5	36.13	0.20	5	37.47	0.59	5	0.99	0.20	5	36.82	0.36	34	38.39	0.74	34	1.58	0.59	34
	8	36.65	0.72	20	37.74	0.14	5	1.19	0.14	5	36.51	0.25	5	37.44	0.24	5	0.89	0.22	5	37.42	0.38	34	38.18	0.32	34	0.72	0.34	34
	13	36.33	0.59	20	38.12	0.03	5	1.77	0.89	5	36.15	0.11	5	37.56	0.10	5	1.29	0.39	5	37.47	0.67	34	39.27	0.52	34	1.43	0.37	34
	14	36.44	0.40	20	38.13	0.22	5	1.78	0.40	5	36.21	0.11	5	37.64	0.14	5	1.70	0.22	5	36.91	0.59	34	38.58	0.45	34	1.97	0.54	34
	15	36.57	0.25	20	37.99	0.25	5	1.30	0.22	5	36.33	0.09	5	37.96	0.17	5	1.60	0.43	5	37.23	0.37	34	38.89	0.58	34	1.75	0.52	34
	16	36.59	0.45	20	37.53	0.19	5	0.67	0.13	5	36.62	0.18	5	37.35	0.08	5	0.83	0.07	5	37.36	0.28	34	38.47	0.38	34	1.25	0.38	34
BW	2	36.86	0.56	20	39.28	0.02	5	2.75	0.31	5	36.52	0.10	5	39.62	0.06	5	3.10	0.08	5	37.18	0.37	33	39.79	0.11	11	2.07	0.31	11
	3	36.86	0.37	20	38.92	0.09	5	2.38	0.20	5	36.14	0.40	5	38.94	0.21	5	2.63	0.10	5	37.56	0.66	33	39.96	0.43	10	2.17	0.42	10
	4	36.97	0.46	20	38.82	0.10	5	1.83	0.10	5	36.98	0.47	5	39.11	0.41	5	2.13	0.08	5	38.04	0.68	33	39.59	0.26	10	1.84	0.43	10
	5	36.65	0.34	20	38.77	0.09	5	2.14	0.04	5	36.59	0.43	5	38.88	0.11	5	2.24	0.33	5	37.63	0.43	33	39.67	0.28	11	1.99	0.35	11
	9	36.58	0.27	20	38.86	0.11	5	2.30	0.38	5	36.89	0.17	5	39.07	0.29	5	2.48	0.13	5	37.35	0.46	33	39.47	0.42	33	2.12	0.48	33
	10	36.97	0.44	20	38.68	0.15	5	1.50	0.21	5	36.76	0.07	5	39.08	0.13	5	2.36	0.27	5	37.68	0.34	33	39.69	0.39	33	1.99	0.41	33
	11	36.40	0.43	20	38.87	0.10	5	2.34	0.28	5	36.19	0.12	5	38.66	0.12	5	2.60	0.08	5	36.97	0.54	33	39.38	0.31	33	2.36	0.48	33
	12	36.83	0.42	20	38.97	0.10	5	2.03	0.31	5	36.57	0.28	5	39.23	0.04	5	2.33	0.27	5	37.86	0.44	33	39.84	0.30	33	2.03	0.26	33
HN	21	36.78	0.40	20	37.93	0.07	5	1.11	0.31	5	36.76	0.16	5	37.94	0.07	5	1.06	0.32	5	37.55	0.35	34	38.85	0.60	34	1.21	0.29	34
	22	36.63	0.39	20	37.84	0.20	5	1.25	0.04	5	36.83	0.14	5	37.64	0.43	5	0.89	0.07	5	37.94	0.48	34	39.28	0.67	34	1.49	0.52	34
	23	37.20	0.15	20	38.32	0.21	5	0.89	0.26	5	37.19	0.02	5	38.06	0.20	5	0.70	0.38	5	38.09	0.33	34	39.11	0.52	34	0.99	0.29	34
	24	36.69	0.21	20	38.09	0.15	5	1.40	0.07	5	36.22	0.29	5	37.86	0.09	5	1.58	0.29	5	37.82	0.34	34	38.99	0.65	34	1.06	0.46	34
	25	37.20	0.23	20	37.56	0.07	5	0.49	0.16	5	37.05	0.12	5	37.53	0.03	5	0.44	0.24	5	37.61	0.34	34	38.25	0.34	34	0.62	0.27	34
	29	37.00	0.31	20	37.77	0.08	5	0.46	0.19	5	36.46	0.24	5	37.66	0.16	5	0.91	0.08	5	37.95	0.50	34	39.08	0.64	34	1.26	0.4	34
	31	37.27	0.26	20	38.19	0.10	5	1.28	0.11	5	37.16	0.12	5	38.29	0.22	5	0.87	0.07	5	37.57	0.45	34	38.66	0.71	34	1.21	0.57	34
	32	36.98	0.21	20	37.77	0.16	5	0.58	0.10	5	36.99	0.03	5	37.69	0.04	5	0.72	0.02	5	37.64	0.27	34	38.83	0.59	34	1.14	0.24	34
HW	17	36.75	0.33	20	38.79	0.07	5	2.06	0.20	5	36.38	0.24	5	38.86	0.10	5	2.41	0.34	5	37.36	0.30	33	39.68	0.49	19	2.24	0.6	19
	18	36.97	0.31	20	38.64	0.17	5	1.71	0.26	5	36.63	0.25	5	38.74	0.07	5	2.11	0.17	5	37.97	0.56	33	39.54	0.20	19	1.48	0.63	19
	19	36.50	0.32	20	38.83	0.28	5	2.56	0.34	5	36.58	0.18	5	38.97	0.09	5	2.47	0.13	5	37.17	0.40	33	39.25	0.18	12	1.87	0.63	12
	20	37.36	0.30	20	39.06	0.18	5	1.47	0.33	5	37.23	0.06	5	39.36	0.16	5	2.13	0.15	5	38.15	0.53	33	39.73	0.48	12	1.82	0.35	12
	26	37.05	0.30	20	39.09	0.27	5	2.33	0.25	5	36.68	0.24	5	39.22	0.07	5	2.36	0.24	5	37.95	0.38	33	39.83	0.27	28	2.07	0.41	28
	27	36.91	0.28	20	38.55	0.04	5	1.62	0.03	5	36.51	0.16	5	38.81	0.15	5	2.10	0.10	5	37.88	0.38	33	39.36	0.30	33	1.63	0.35	33
	28	36.79	0.43	20	39.14	0.09	5	2.16	0.12	5	36.65	0.29	5	39.01	0.20	5	2.43	0.54	5	38.36	0.73	33	40.01	0.54	33	1.97	0.41	33
	30	37.34	0.16	20	38.89	0.11	5	1.39	0.11	5	37.55	0.25	5	39.29	0.61	5	1.77	0.13	5	38.19	0.31	33	40.02	0.55	28	1.89	0.28	28
Control	33	37.46	0.16	20	38.88	0.05	5	1.46	0.05	5	36.99	0.13	5	39.39	0.08	3	2.47	0.07	3	37.07	0.21	33	39.04	0.10	31	1.95	0.31	31
	34	37.30	0.27	20	39.42	0.15	5	1.83	0.16	5	37.24	0.11	5	39.89	0.13	3	2.72	0.09	3	36.90	0.41	33	39.54	0.21	31	2.69	0.41	31

38. Weekly mean PCV (%) of each animal over the course of the study (n = 3) (Gambia study, Chapter 6).

		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10		Week 11		Week 12	
Group	Animal	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
BN	1	29.5	0.84	28.9	0.63	27.9	0.50	28.0	1.74	27.1	1.07	27.5	0.45	25.9	0.87	23.2	0.73	22.1	0.40	21.0	0.23	20.6	0.78
	6	29.6	0.87	29.5	0.73	28.5	0.68	28.1	0.52	25.6	0.23	23.7	0.45	21.8	0.13	19.1	0.74	18.5	0.95	18.0	0.20	17.2	0.13
	7	34.0	0.81	33.4	0.64	33.2	0.64	34.3	1.35	28.7	1.31	25.4	0.23	24.1	0.33	24.7	0.74	22.8	0.58	24.8	0.32	25.3	1.19
	8	32.9	1.49	33.7	0.23	30.2	0.56	31.8	0.55	27.7	0.65	24.5	0.33	23.1	0.50	22.1	0.37	23.0	0.68	22.5	0.20	20.6	0.64
	13	31.0	0.77	30.3	0.27	30.6	0.20	29.1	0.87	27.6	1.60	24.4	0.27	22.7	0.32	21.5	0.55	20.4	0.55	20.1	0.55	19.8	0.37
	14	30.2	0.64	26.5	0.23	26.9	0.64	25.5	0.52	23.6	1.27	20.6	0.58	20.2	0.37	20.0	0.50	18.4	0.40	18.7	0.13	16.8	0.64
	15	31.2	2.10	28.7	0.43	28.2	0.33	28.4	0.58	26.5	1.52	22.5	0.20	20.4	0.85	20.0	0.50	19.6	0.27	19.1	1.29	19.1	0.77
	16	29.0	0.60	28.6	0.32	27.3	0.58	29.4	0.37	24.0	0.65	24.8	0.23	23.7	0.55	21.7	0.87	21.4	0.58	22.1	0.00	20.6	0.58
BW	2	26.1	0.43	25.4	0.23	25.0	0.43	25.1	0.13	22.7	0.64	20.5	0.55	19.1	0.43	18.7	0.13	18.2	0.23	18.8	0.20	17.2	0.32
	3	27.9	0.32	27.4	0.32	27.3	0.58	26.3	0.50	25.3	0.69	23.5	0.37	21.6	0.64	20.6	0.23	22.6	1.00	22.4	0.37	21.1	0.46
	4	24.4	0.52	23.6	0.20	23.7	0.10	24.1	0.45	22.7	0.50	20.9	0.50	15.9	0.69	14.8	0.32	14.6	0.27	13.7	0.52	14.4	0.65
	5	32.7	0.43	31.6	0.91	30.8	0.45	30.1	0.88	25.5	0.50	22.6	0.81	18.3	1.25	14.7	0.40	14.2	0.32	13.8	0.45	13.2	0.43
	9	33.3	2.57	32.4	0.55	31.4	0.88	29.5	0.43	28.4	0.20	25.4	0.40	22.9	1.28	22.1	1.10	21.4	0.20	21.0	0.43	20.9	0.52
	10	28.0	0.23	27.3	0.43	28.4	1.20	27.0	0.60	25.5	1.44	24.9	0.10	22.3	0.47	20.4	0.50	18.5	0.43	18.5	0.65	16.7	0.32
	11	30.8	0.32	28.6	1.10	29.2	0.43	27.6	1.00	24.9	0.81	23.0	0.47	21.3	0.13	19.3	0.47	19.0	0.10	18.6	0.23	17.2	0.65
	12	33.3	1.19	32.2	1.60	33.3	1.37	30.0	1.00	27.6	0.37	26.4	0.46	23.2	1.17	19.6	0.32	20.1	1.00	18.4	0.23	17.8	0.55
HN	21	31.6	0.13	30.5	0.13	31.7	0.97	32.0	0.58	28.6	1.19	27.0	0.55	24.3	0.00	23.0	0.45	22.5	0.43	22.8	0.20	22.5	0.74
	22	28.9	1.42	28.3	0.52	26.9	1.13	27.6	1.00	26.6	0.88	24.8	0.10	23.7	0.32	21.5	0.75	19.1	0.20	20.1	0.45	20.9	0.35
	23	30.3	2.27	29.0	1.05	27.6	0.58	28.0	0.91	25.9	1.37	23.1	0.88	21.7	0.87	19.1	0.00	19.0	0.32	18.3	0.60	16.9	0.43
	24	30.0	0.23	27.5	0.32	27.7	0.82	28.0	0.64	25.7	0.75	23.7	1.05	21.2	1.00	18.5	0.27	18.5	0.69	18.6	0.33	18.6	0.63
	25	32.7	0.27	29.3	0.45	29.6	0.60	29.1	0.56	27.4	0.67	26.1	0.58	23.3	0.43	19.9	0.20	19.7	0.68	19.9	0.77	20.2	0.77
	29	28.9	0.33	28.4	0.55	27.5	0.32	28.1	0.65	25.3	0.81	21.4	0.43	20.5	0.32	18.6	0.45	19.7	1.00	18.3	0.75	16.8	0.50
	31	39.2	1.29	37.7	1.18	37.7	0.32	37.2	0.92	33.3	0.81	27.9	0.99	24.5	0.79	25.6	0.33	23.7	0.60	23.6	1.85	24.3	0.40
	32	31.4	1.04	30.1	0.50	29.1	0.73	28.1	0.27	27.5	0.87	24.4	0.27	23.0	0.45	20.4	0.33	19.0	0.50	19.3	0.79	19.3	0.33
HW	17	31.8	0.87	31.4	0.88	30.6	0.64	30.4	1.45	26.8	1.10	23.9	0.20	20.5	0.13	19.9	0.58	18.4	0.23	19.6	0.52	20.9	0.13
	18	30.2	0.56	30.1	0.75	28.8	0.13	29.5	0.00	25.3	1.04	23.2	0.78	21.0	1.00	18.0	0.58	17.1	0.33	18.3	0.87	19.0	0.64
	19	30.2	1.00	30.3	0.52	30.3	0.43	30.2	0.56	24.6	1.39	22.1	0.40	19.0	0.95	17.4	0.43	16.9	0.43	16.8	0.87	16.2	0.43
	20	24.9	0.87	24.4	0.83	23.4	0.45	24.9	0.23	21.5	1.10	20.0	0.45	16.9	0.64	14.0	0.20	14.2	0.10	14.6	0.75	15.6	0.63
	26	35.0	0.91	34.6	0.64	35.0	1.80	33.9	1.04	30.8	0.67	29.6	0.85	26.2	0.58	22.7	0.32	20.6	0.43	19.8	0.13	17.2	0.27
	27	30.7	1.27	28.8	0.52	28.3	0.52	28.0	0.23	26.6	0.27	24.7	0.58	23.0	0.23	21.5	0.32	21.1	0.87	19.5	0.23	19.5	0.37
	28	30.3	1.39	30.3	0.75	30.4	0.47	30.8	0.67	27.4	1.19	26.1	0.77	25.0	0.20	23.0	0.33	21.0	0.56	19.4	0.13	20.4	0.23
	30	30.2	1.17	27.9	0.50	28.9	0.63	26.4	0.13	24.0	0.52	22.2	0.13	21.1	0.55	19.2	0.37	17.8	0.55	16.9	0.58	17.8	0.85
Control	33	29.3	0.45	29.1	0.73	29.5	0.43	28.3	0.65	27.6	0.64	26.8	0.65	26.3	0.33	25.3	0.43	26.1	0.77	25.9	0.71	25.0	0.55
	34	27.4	0.75	28.1	0.83	28.6	0.50	26.0	1.42	23.6	0.97	22.8	0.84	23.2	0.97	23.7	0.32	25.0	0.00	26.3	0.63	26.0	0.23

39. Weekly mean white blood cell (WBC) count (x 10^9 L^{-1}) of each animal over the course of the study (n = 3), (Gambia study, Chapter 6).

		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10		Week 11		Week 12	
Group	Animal	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
BN	1	16.2	0.70	16.1	0.35	18.7	0.70	16.3	2.00	8.7	0.70	9.6	0.48	12.7	0.96	14.3	1.26	12.0	0.39	10.4	0.99	8.9	0.81
	6	10.8	0.58	10.2	0.32	10.5	0.46	10.3	0.35	6.2	0.47	6.1	0.15	7.3	1.17	7.6	0.37	6.9	0.67	6.4	0.57	5.1	0.23
	7	14.3	0.40	13.0	0.44	15.9	0.41	16.0	0.80	9.6	0.85	9.4	1.17	11.1	0.44	12.3	0.58	11.5	0.73	10.5	0.37	11.9	0.81
	8	15.2	0.09	15.7	1.77	13.6	1.20	17.3	1.21	8.9	0.79	8.6	0.69	12.4	0.80	13.0	0.43	14.4	1.01	11.5	0.71	11.6	0.92
	13	13.7	0.52	13.9	0.20	15.5	0.72	13.4	1.12	7.8	1.35	6.8	0.35	9.4	0.22	9.8	0.15	9.1	0.35	8.1	0.67	8.3	0.37
	14	10.0	0.32	9.3	0.28	10.7	1.09	9.3	0.38	7.8	1.07	8.6	0.90	10.0	0.21	12.8	0.32	11.9	1.12	13.6	0.58	10.1	0.38
	15	10.2	0.17	10.1	0.29	9.7	0.26	9.8	0.32	5.8	0.78	6.1	0.12	8.3	0.10	10.8	0.49	10.3	0.39	8.9	0.50	7.7	0.50
	16	10.7	0.09	11.0	0.12	12.3	1.18	11.2	0.54	6.8	0.15	8.6	0.55	11.0	0.63	11.7	0.53	11.0	0.47	10.7	1.10	11.5	0.72
BW	2	11.4	0.21	11.9	0.07	11.9	0.28	9.9	0.95	5.7	0.15	11.0	0.74	10.6	0.85	12.6	0.19	11.2	0.91	11.1	0.88	9.8	0.17
	3	13.6	0.90	13.0	1.72	12.9	0.65	10.0	1.15	6.5	0.54	6.2	0.12	8.6	0.94	15.2	1.04	17.0	0.12	14.5	1.27	11.5	0.25
	4	10.1	0.03	10.1	0.00	10.4	0.33	8.9	1.36	4.4	0.60	4.1	0.40	6.4	0.43	11.2	0.38	7.5	0.48	8.1	1.03	6.2	0.55
	5	16.6	0.47	16.1	0.15	17.6	0.44	15.9	0.63	9.7	0.12	9.1	0.75	9.1	0.71	11.4	0.96	10.2	1.12	10.0	0.83	11.9	0.58
	9	12.1	0.59	12.6	0.26	13.4	0.71	11.9	1.37	7.7	1.01	6.2	0.26	8.1	0.55	9.7	0.58	8.5	0.91	8.2	0.03	7.5	0.69
	10	14.7	0.53	15.0	0.12	16.7	0.52	14.0	1.65	8.5	1.07	9.2	0.06	10.5	0.20	13.4	0.33	12.5	0.64	13.0	0.70	12.6	0.50
	11	12.5	0.52	12.4	0.15	15.3	0.57	12.9	0.92	6.5	0.64	7.5	0.49	8.0	0.40	10.1	0.37	8.2	0.66	7.4	0.47	7.6	0.29
	12	10.9	0.74	12.0	0.07	13.5	0.15	11.5	0.98	6.5	0.18	7.9	0.55	7.5	0.15	7.8	0.32	7.0	0.48	7.2	0.32	7.2	0.68
HN	21	18.9	0.12	18.5	0.46	19.1	1.04	18.1	0.71	11.1	0.98	10.9	0.18	12.7	0.52	13.8	0.28	12.7	0.32	12.5	0.35	12.4	1.70
	22	12.4	0.61	11.9	0.27	12.2	0.59	11.1	1.89	7.0	0.48	7.3	0.69	10.6	0.39	12.7	0.19	11.7	0.54	11.3	0.81	11.9	1.27
	23	14.2	0.34	14.3	0.38	13.9	0.23	14.4	0.12	8.9	0.62	7.2	0.22	9.5	1.52	10.6	0.24	9.7	0.27	8.2	0.36	7.6	0.71
	24	10.8	0.12	10.3	0.21	11.1	0.61	10.8	0.67	6.8	1.15	5.8	0.15	7.0	0.21	7.9	0.40	7.9	0.40	7.3	0.46	6.1	0.44
	25	10.8	0.25	10.3	0.20	11.6	0.58	12.4	0.69	6.4	0.22	5.3	0.10	6.2	0.23	6.8	0.49	6.2	0.38	5.8	0.07	5.9	0.03
	29	9.7	0.27	9.7	0.24	10.9	0.29	10.3	0.68	6.6	1.19	5.3	0.20	6.2	0.10	6.1	0.33	6.5	0.07	6.4	0.59	6.3	0.42
	31	12.8	0.09	12.3	0.31	13.0	0.29	13.9	0.55	7.2	0.76	5.4	0.34	7.3	1.21	9.6	0.68	8.5	0.26	7.7	0.52	8.7	0.57
	32	12.6	0.63	12.5	1.42	14.2	0.41	15.3	0.18	9.4	0.70	8.2	0.07	10.6	1.17	11.3	0.34	9.8	0.38	9.1	0.32	8.9	0.55
HW	17	14.1	0.18	15.5	0.59	16.4	0.72	12.9	2.76	8.1	0.70	6.6	0.30	7.4	0.18	8.7	0.81	9.3	0.70	8.4	0.12	10.5	0.94
	18	10.3	0.32	10.3	0.12	10.7	0.45	10.7	0.12	5.1	0.54	5.3	0.18	6.5	0.41	8.5	0.70	7.6	0.15	7.4	0.32	7.2	0.35
	19	13.3	0.42	13.4	0.23	14.3	0.38	13.4	0.83	5.5	0.24	7.2	0.35	8.1	0.21	8.2	0.13	8.3	0.31	8.4	0.31	8.5	0.40
	20	16.0	1.79	19.7	0.55	18.9	0.58	16.0	1.54	7.1	1.57	6.9	0.42	9.8	1.09	10.5	0.94	10.9	0.68	11.7	0.52	10.4	1.23
	26	10.6	0.62	11.3	0.33	12.1	0.35	11.8	1.10	7.0	1.01	5.2	0.15	6.6	0.60	7.2	0.59	6.3	0.49	5.5	0.39	5.6	0.48
	27	12.1	1.11	12.5	0.13	12.4	0.29	12.1	0.45	7.2	0.61	7.0	0.12	7.0	0.26	8.3	0.12	9.5	0.43	9.0	0.43	10.1	0.38
	28	10.4	0.43	10.3	0.10	11.3	0.43	10.8	0.38	6.1	0.48	5.8	0.52	8.0	0.73	10.1	0.18	12.4	0.82	9.9	0.40	9.6	0.75
	30	11.9	0.38	10.6	0.39	11.0	0.42	7.1	0.57	7.8	0.06	7.1	0.03	11.4	1.06	12.0	1.17	8.9	0.42	8.6	0.55	10.4	0.38
Control	33	11.6	0.87	10.7	0.33	11.7	0.51	11.4	0.37	11.2	0.32	10.5	0.29	10.4	0.44	10.5	0.19	10.4	0.31	9.9	0.19	9.8	0.03
	34	12.8	0.22	13.5	0.41	13.0	0.78	15.8	1.08	12.7	0.19	13.1	0.38	12.4	0.20	11.3	0.10	11.6	0.12	11.0	0.30	11.1	0.15

40. Weekly mean red blood cell (RBC) count (x 10^{12} L^{-1}) of each animal over the course of the study (n = 3), (Gambia study, Chapter 6).

		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10		Week 11		Week 12	
Group	Animal	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
BN	1	6.8	0.18	6.7	0.13	6.4	0.10	6.4	0.39	6.2	0.24	6.3	0.11	5.9	0.20	5.2	0.17	4.9	0.07	4.6	0.02	4.5	0.15
	6	7.1	0.22	7.1	0.19	6.8	0.17	6.7	0.13	6.0	0.08	5.6	0.10	5.1	0.01	4.3	0.18	4.1	0.21	3.8	0.07	3.4	0.02
	7	8.5	0.21	8.4	0.16	8.3	0.14	8.5	0.35	7.0	0.31	6.2	0.05	5.9	0.09	5.9	0.20	5.4	0.12	5.8	0.10	5.8	0.27
	8	8.1	0.35	8.2	0.07	7.4	0.15	7.7	0.12	6.6	0.16	5.9	0.11	5.5	0.12	5.2	0.11	5.3	0.15	5.2	0.06	4.8	0.16
	13	8.0	0.18	7.8	0.06	7.8	0.02	7.3	0.21	6.7	0.39	5.9	0.05	5.4	0.10	5.0	0.15	4.6	0.15	4.4	0.14	4.2	0.10
	14	7.4	0.13	6.4	0.05	6.5	0.15	6.2	0.13	5.6	0.28	4.9	0.11	4.8	0.10	4.7	0.14	4.3	0.10	4.2	0.05	3.7	0.13
	15	7.9	0.56	7.2	0.11	7.1	0.11	7.0	0.18	6.4	0.38	5.4	0.04	4.8	0.21	4.6	0.11	4.3	0.07	4.1	0.26	4.0	0.17
	16	6.0	0.14	5.9	0.08	5.6	0.11	6.0	0.09	4.8	0.13	5.0	0.06	4.8	0.12	4.4	0.16	4.3	0.11	4.4	0.03	4.0	0.10
BW	2	6.3	0.10	6.1	0.04	5.9	0.11	5.8	0.02	5.2	0.17	4.7	0.10	4.3	0.12	4.0	0.05	3.8	0.05	3.8	0.03	3.4	0.06
	3	7.3	0.06	7.1	0.06	7.0	0.13	6.7	0.11	6.4	0.15	6.0	0.12	5.5	0.16	5.1	0.05	5.5	0.23	5.5	0.09	5.1	0.14
	4	6.0	0.15	5.8	0.04	5.7	0.04	5.8	0.11	5.4	0.10	4.8	0.16	3.5	0.24	2.7	0.06	2.5	0.03	2.3	0.09	2.4	0.10
	5	7.4	0.08	7.2	0.21	7.0	0.10	6.8	0.20	5.8	0.10	5.1	0.18	4.2	0.27	3.1	0.02	2.8	0.06	2.5	0.05	2.3	0.07
	9	8.8	0.67	8.5	0.17	8.3	0.23	7.7	0.12	7.3	0.07	6.6	0.11	5.9	0.35	5.6	0.28	5.4	0.06	5.2	0.11	5.1	0.12
	10	6.2	0.04	5.9	0.09	6.1	0.28	5.8	0.15	5.4	0.31	5.3	0.04	4.8	0.12	4.3	0.10	3.8	0.10	3.7	0.13	3.3	0.08
	11	8.2	0.12	7.5	0.29	7.6	0.12	7.1	0.23	6.3	0.23	5.7	0.11	5.2	0.03	4.6	0.12	4.4	0.03	4.2	0.04	3.8	0.14
	12	8.6	0.33	8.2	0.40	8.4	0.35	7.4	0.27	6.8	0.08	6.4	0.12	5.6	0.31	4.5	0.08	4.5	0.27	4.0	0.03	3.8	0.09
HN	21	8.0	0.07	7.7	0.02	7.9	0.29	7.9	0.14	7.0	0.30	6.6	0.14	5.8	0.02	5.4	0.12	5.1	0.08	5.1	0.04	4.9	0.15
	22	7.2	0.36	7.0	0.12	6.7	0.30	6.8	0.24	6.6	0.21	6.1	0.05	5.9	0.06	5.2	0.19	4.5	0.06	4.7	0.10	4.7	0.06
	23	7.6	0.59	7.2	0.26	6.9	0.15	6.9	0.22	6.3	0.35	5.6	0.21	5.3	0.23	4.4	0.02	4.4	0.07	4.1	0.14	3.8	0.12
	24	7.1	0.06	6.5	0.08	6.5	0.19	6.5	0.14	5.8	0.19	5.3	0.21	4.7	0.22	4.0	0.08	3.8	0.16	3.7	0.09	3.6	0.13
	25	8.3	0.06	7.4	0.13	7.4	0.16	7.2	0.11	6.7	0.15	6.4	0.13	5.6	0.07	4.7	0.05	4.6	0.15	4.4	0.14	4.3	0.18
	29	7.1	0.09	7.0	0.12	6.7	0.07	6.8	0.16	6.1	0.18	5.1	0.11	4.8	0.07	4.2	0.12	4.4	0.27	3.9	0.16	3.5	0.11
	31	9.3	0.28	8.9	0.28	8.9	0.07	8.7	0.21	7.7	0.18	6.4	0.23	5.6	0.20	5.6	0.08	5.1	0.15	4.9	0.40	4.9	0.12
	32	7.7	0.28	7.4	0.14	7.1	0.16	6.8	0.05	6.6	0.22	5.8	0.04	5.4	0.10	4.7	0.08	4.3	0.09	4.2	0.16	4.1	0.05
HW	17	8.5	0.26	8.3	0.23	8.0	0.16	7.8	0.35	6.7	0.28	5.9	0.07	4.9	0.06	4.5	0.17	4.0	0.07	4.1	0.09	4.3	0.04
	18	7.7	0.14	7.6	0.21	7.2	0.04	7.3	0.02	6.1	0.27	5.5	0.17	4.9	0.24	4.0	0.17	3.4	0.02	3.5	0.18	3.6	0.11
	19	7.5	0.24	7.5	0.17	7.5	0.13	7.4	0.16	6.0	0.32	5.2	0.05	4.3	0.24	3.7	0.14	3.4	0.10	3.3	0.18	3.1	0.07
	20	6.0	0.19	6.0	0.21	5.8	0.13	6.2	0.07	5.3	0.27	4.9	0.10	4.1	0.17	3.1	0.04	2.9	0.05	2.9	0.17	3.0	0.11
	26	8.6	0.22	8.4	0.15	8.4	0.45	8.0	0.26	7.2	0.17	6.9	0.19	6.1	0.13	5.0	0.12	4.4	0.08	4.0	0.02	3.3	0.07
	27	7.9	0.28	7.3	0.13	7.1	0.18	6.9	0.02	6.5	0.05	5.9	0.12	5.5	0.08	5.1	0.09	4.8	0.20	4.3	0.09	4.0	0.05
	28	7.1	0.30	7.0	0.16	7.0	0.12	7.0	0.13	6.1	0.26	5.9	0.20	5.6	0.06	5.2	0.08	4.7	0.14	4.2	0.02	4.3	0.08
	30	7.6	0.28	7.0	0.13	7.2	0.15	6.5	0.02	5.8	0.15	5.2	0.02	4.7	0.15	3.9	0.08	3.5	0.12	3.2	0.15	3.1	0.17
Control	33	7.9	0.14	7.8	0.20	7.9	0.11	7.5	0.18	7.3	0.20	7.0	0.15	6.8	0.08	6.5	0.11	6.6	0.19	6.4	0.19	6.1	0.14
	34	6.1	0.15	6.2	0.21	6.3	0.09	5.7	0.28	5.2	0.24	4.9	0.19	4.9	0.21	4.9	0.05	5.1	0.01	5.3	0.13	5.2	0.07

41. Weekly mean haemoglobin level (g/dL) of each animal over the course of the study (n = 3), (Gambia study, Chapter 6).

		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8		Week 9		Week 10		Week 11		Week 12	
Group	Animal	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
BN	1	9.9	0.30	9.7	0.22	9.1	0.15	9.9	0.52	9.2	0.20	9.6	0.17	8.9	0.30	7.9	0.25	7.6	0.12	7.1	0.03	7.1	0.15
	6	9.6	0.29	9.5	0.28	9.1	0.19	9.2	0.18	8.5	0.07	7.9	0.15	7.2	0.00	6.3	0.29	6.1	0.35	5.9	0.09	5.5	0.06
	7	11.3	0.28	11.0	0.15	10.9	0.12	11.5	0.50	9.4	0.35	8.6	0.09	8.1	0.17	8.3	0.38	7.6	0.15	8.1	0.06	8.3	0.38
	8	10.7	0.47	11.1	0.18	9.8	0.23	10.5	0.27	9.1	0.20	8.2	0.15	7.7	0.20	7.4	0.22	7.7	0.18	7.4	0.12	6.8	0.17
	13	10.1	0.19	9.7	0.09	9.6	0.19	9.3	0.23	8.9	0.47	7.9	0.09	7.4	0.15	7.1	0.18	6.8	0.17	6.6	0.15	6.5	0.12
	14	9.9	0.23	8.7	0.12	8.6	0.09	8.2	0.20	7.7	0.35	6.7	0.17	6.6	0.10	6.6	0.23	5.9	0.12	5.8	0.03	5.3	0.22
	15	10.1	0.72	9.3	0.13	8.9	0.12	9.1	0.12	8.4	0.42	7.2	0.12	6.5	0.33	6.6	0.20	6.4	0.09	6.2	0.40	6.0	0.28
	16	9.6	0.18	9.6	0.12	8.9	0.12	9.8	0.12	8.0	0.23	8.2	0.15	8.0	0.12	7.4	0.26	7.1	0.13	7.4	0.09	6.7	0.12
BW	2	8.3	0.10	8.2	0.13	7.9	0.09	8.1	0.09	7.3	0.09	6.7	0.15	6.2	0.15	6.2	0.10	6.0	0.06	6.0	0.07	5.5	0.09
	3	8.8	0.06	8.8	0.09	8.5	0.09	8.4	0.18	8.1	0.09	7.7	0.09	6.9	0.21	6.7	0.06	7.2	0.24	7.0	0.09	6.6	0.12
	4	7.4	0.15	7.4	0.03	7.2	0.03	7.5	0.09	7.1	0.12	6.6	0.24	4.9	0.22	4.6	0.15	4.6	0.09	4.2	0.23	4.5	0.26
	5	11.2	0.12	10.8	0.30	10.4	0.18	10.2	0.26	8.7	0.21	7.7	0.25	6.2	0.37	5.0	0.15	4.9	0.09	4.6	0.19	4.5	0.15
	9	11.0	0.87	10.8	0.32	10.2	0.46	9.7	0.15	9.3	0.07	8.4	0.15	7.6	0.46	7.4	0.40	7.1	0.06	6.9	0.10	6.9	0.12
	10	9.3	0.09	8.9	0.15	9.2	0.37	8.9	0.25	8.3	0.45	8.1	0.06	7.3	0.15	6.9	0.18	6.2	0.23	5.9	0.26	5.3	0.12
	11	9.9	0.15	9.0	0.44	9.0	0.03	8.8	0.35	7.9	0.30	7.3	0.17	6.7	0.07	6.1	0.19	6.0	0.06	5.8	0.03	5.4	0.17
	12	11.1	0.54	10.7	0.57	10.9	0.38	9.8	0.24	9.2	0.00	8.7	0.13	7.7	0.39	6.4	0.20	6.7	0.35	5.9	0.10	5.6	0.19
HN	21	10.5	0.15	10.1	0.10	10.3	0.38	10.7	0.23	9.6	0.32	9.0	0.20	8.1	0.13	7.9	0.15	7.7	0.10	7.8	0.06	7.5	0.29
	22	9.6	0.53	9.3	0.24	8.6	0.30	9.1	0.33	8.7	0.25	8.0	0.00	7.8	0.12	7.1	0.23	6.3	0.15	6.5	0.07	6.7	0.09
	23	9.8	0.80	9.4	0.35	8.8	0.15	9.1	0.30	8.4	0.47	7.4	0.34	7.1	0.23	6.3	0.03	6.4	0.13	5.7	0.22	5.3	0.12
	24	10.0	0.17	9.2	0.03	9.0	0.28	9.3	0.25	8.5	0.37	7.8	0.36	7.0	0.32	6.1	0.15	6.1	0.22	5.9	0.06	6.0	0.20
	25	10.7	0.03	9.3	0.15	9.3	0.13	9.1	0.15	8.7	0.35	8.4	0.25	7.4	0.10	6.5	0.07	6.4	0.23	6.2	0.23	6.3	0.32
	29	9.5	0.03	9.5	0.12	8.8	0.07	9.3	0.19	8.3	0.27	6.9	0.13	6.7	0.03	6.0	0.13	6.4	0.32	5.7	0.15	5.3	0.20
	31	13.3	0.23	12.7	0.49	12.2	0.12	12.5	0.43	11.1	0.27	9.2	0.35	8.0	0.30	8.6	0.09	8.0	0.17	7.7	0.56	7.8	0.09
	32	10.3	0.39	10.0	0.24	9.3	0.20	9.2	0.13	9.0	0.32	7.9	0.09	7.5	0.15	6.6	0.24	6.1	0.22	5.9	0.20	6.0	0.09
HW	17	10.4	0.32	10.4	0.41	9.8	0.23	10.0	0.49	8.6	0.27	7.7	0.09	6.6	0.09	6.4	0.20	6.0	0.07	6.3	0.20	6.7	0.03
	18	9.8	0.20	9.8	0.29	9.2	0.07	9.6	0.09	8.2	0.37	7.5	0.13	6.8	0.35	5.7	0.15	5.4	0.12	5.8	0.29	6.1	0.18
	19	9.9	0.18	9.9	0.19	9.7	0.10	9.9	0.12	7.9	0.41	7.1	0.09	6.1	0.32	5.5	0.12	5.4	0.15	5.2	0.23	5.0	0.15
	20	8.0	0.15	8.1	0.34	7.3	0.19	7.8	0.09	6.7	0.28	6.3	0.15	5.4	0.19	4.5	0.06	4.6	0.06	4.6	0.20	4.8	0.12
	26	11.9	0.44	11.7	0.21	11.4	0.55	11.4	0.38	10.3	0.23	9.8	0.35	8.7	0.33	7.6	0.09	6.8	0.18	6.5	0.09	5.5	0.09
	27	9.9	0.31	9.5	0.15	8.9	0.18	9.1	0.12	8.6	0.12	7.8	0.15	7.4	0.12	7.0	0.12	6.8	0.15	5.9	0.07	6.0	0.18
	28	10.1	0.44	10.1	0.26	9.8	0.17	10.2	0.26	9.0	0.27	8.8	0.23	8.4	0.13	7.7	0.09	7.2	0.21	6.4	0.03	6.7	0.09
	30	9.5	0.23	9.0	0.09	9.0	0.15	8.4	0.09	7.4	0.09	7.0	0.03	6.7	0.17	6.1	0.20	5.8	0.27	5.2	0.12	5.4	0.30
Control	33	9.6	0.15	9.5	0.25	9.4	0.15	9.2	0.12	8.9	0.15	8.7	0.10	8.7	0.06	8.4	0.12	8.7	0.29	8.4	0.30	8.0	0.19
	34	9.0	0.15	9.2	0.28	9.1	0.17	8.3	0.42	7.6	0.35	7.3	0.32	7.5	0.30	7.8	0.13	8.4	0.09	8.6	0.12	8.5	0.10

42. Individual animal IgG_1 responses to infection measured by ELISA (OD values) without correction for plate to plate variation, (Gambia study, Chapter 6).

(Missing values indicate where the OD's of duplicate wells differed by more than 10% of the anti-log10 of the larger value.)

Plate No.		33	34	35	30	37	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Day of the study		1	3	5	24	26	29	31	33	36	38	40	43	45	47	50	52	54	57	59	61	64	66	68	71	73	75	78	80	82	85
Group	Bull																														
BN	1	0.2650	0.3060		0.0460	0.0465	0.0820	0.0935	0.1060	0.1160	0.0650	0.0625	0.0955	0.0990	0.0810	0.0705	0.0985	0.0720	0.0700	0.0825	0.1255	0.1080	0.1470	0.1960	0.1910	0.1345	0.2185	0.2805	0.2025	0.2415	0.2460
	6	0.0680	0.0595	0.0575	0.0485	0.0440	0.0640	0.0695	0.0790	0.1040	0.0540	0.0695	0.0740	0.0715	0.0590	0.0560	0.0655	0.0540	0.0580	0.0555	0.1060	0.0895	0.0860	0.0985	0.1050	0.0895	0.1070	0.1305	0.1185	0.1280	0.1120
	7	0.0925	0.0880	0.0785	0.0420	0.0525	0.0945	0.1010	0.1045	0.1210	0.0845	0.0840	0.1025	0.1155	0.0885	0.0720	0.1150	0.0780	0.0725	0.0780	0.1230	0.0925	0.1225	0.1280	0.1460	0.1290	0.1225	0.1565	0.1205	0.1160	0.1165
	8	0.0855	0.0705	0.0595	0.0590	0.0530	0.0665	0.0680	0.0890	0.1040	0.0670	0.0740	0.0815	0.0830	0.0675	0.0620	0.0930	0.0645	0.0685	0.0840	0.1560	0.1540	0.1825	0.2340	0.2170	0.1860	0.2345	0.2910	0.2140	0.2355	0.2620
	13	0.0800	0.0750	0.0705	0.0550	0.0525	0.1125	0.1080	0.1160	0.1295	0.0725	0.0755	0.0885	0.0920	0.0760	0.0695	0.0770	0.0685	0.0620	0.0735	0.1140	0.0925	0.0945	0.1045	0.0780	0.0790	0.0945	0.0990	0.0815	0.0800	0.0705
	14	0.0585	0.0625	0.0630	0.0445	0.0460	0.0955	0.0980	0.0965	0.1245	0.0705	0.0745	0.0930	0.0915	0.0730	0.0710	0.0770	0.0645	0.0770	0.0705	0.1210	0.0940	0.1140	0.1180	0.1025	0.1105	0.1040	0.1135	0.1005	0.0885	0.1125
	15	0.0715	0.0790	0.0745	0.0530	0.0610	0.0825	0.0830	0.0945	0.1085	0.0680	0.0730	0.0960	0.1115	0.0835	0.0770	0.1060	0.0825	0.0790	0.0755	0.1715	0.1540	0.1700	0.2065	0.1580	0.1695	0.1625	0.1755	0.1465	0.1535	0.1295
	16	0.0590	0.0550	0.0495	0.0480	0.0510	0.0640	0.0690	0.0785	0.0960	0.0515	0.0570	0.0585	0.0600	0.0530	0.0475	0.0575	0.0525	0.0510	0.0525	0.0645	0.0650	0.0760	0.0915	0.0865	0.0990	0.0925	0.1105	0.0820	0.0835	0.1100
BW	2	0.1735	0.1835	0.1585	0.0935	0.1275	0.2180	0.2045	0.1990	0.2385	0.1530	0.1450	0.1980	0.1820	0.1155	0.1100	0.1455	0.1195	0.0950	0.1060	0.2215	0.1770	0.1900	0.2130	0.1890	0.2045	0.2060	0.2170	0.2200	0.2275	0.2210
	3	0.0625	0.0645	0.0600	0.0385	0.0410	0.0755	0.0710	0.0875	0.0960	0.0605	0.0790	0.1130	0.1300	0.0865	0.0555	0.1010	0.1020	0.0675	0.0705	0.1885	0.1490	0.1815	0.2090	0.2130	0.1905	0.2475	0.2740	0.2280	0.2290	0.2545
	4	0.0655	0.0570	0.0545	0.0395	0.0510	0.0625	0.0745	0.0850	0.1840	0.1715	0.2180	0.2060	0.1605	0.1045	0.0775	0.1045	0.0935	0.0795	0.0805	0.1625	0.1540	0.1975	0.2230	0.1995	0.1870	0.2620	0.3005	0.2015	0.2095	0.2450
	5	0.1395	0.1380	0.1355	0.0775	0.1055	0.3030		0.2545	0.1850	0.1010	0.1265	0.1530	0.1505	0.0915	0.0800	0.0915	0.0680	0.0830	0.0910	0.1720	0.1655	0.1700	0.1705	0.1245	0.1265	0.1590	0.2030	0.1480	0.1750	0.1765
	9	0.0685	0.0675	0.0735	0.0490	0.0440	0.0935	0.0960	0.1075	0.1250	0.0745	0.0875	0.1100	0.1210	0.0855	0.0745	0.0930	0.0730	0.0805		0.1320	0.1150	0.1640	0.1625	0.1595	0.1460	0.1600	0.1895	0.1410	0.1235	0.1110
	10	0.0835	0.0845	0.0830	0.0540	0.0560	0.1310	0.1345	0.1130	0.1770	0.0960	0.0955	0.1280	0.1435	0.1090	0.1035	0.1510	0.1155	0.1235	0.1555	0.3480	0.2300	0.2450	0.2530	0.3315	0.3310	0.4135	0.4425	0.4060	0.3915	0.3540
	11	0.0590	0.0435	0.0465	0.0405	0.0435	0.0495	0.0515	0.0645	0.0815	0.0460	0.0545	0.0495	0.0455	0.0475	0.0415	0.0545	0.0455	0.0435	0.0510	0.0530	0.0595	0.0670	0.0775	0.0755	0.0725	0.1070	0.1185	0.1595	0.1380	0.0995
	12	0.0580	0.0610	0.0495	0.0480	0.0505	0.0655	0.0845	0.0860	0.0980	0.0575	0.0625	0.0595	0.0595	0.0510	0.0480	0.0560	0.0490	0.0450	0.0570	0.0725	0.0790	0.0845	0.0910	0.0920	0.0775	0.1290	0.1430	0.1185	0.1300	0.1410
HN	21	0.0785	0.0820	0.0830	0.0455	0.0575	0.1100	0.1090	0.1330	0.1645	0.1130	0.1275	0.1705	0.1820	0.1240	0.1145	0.1275	0.0730	0.0840	0.0900	0.1560	0.1345	0.1405	0.1650	0.1395	0.1235	0.1350	0.1495	0.1300	0.1400	0.1170
	22	0.0495	0.0520	0.0530	0.0425	0.0420	0.0620	0.0645	0.0815	0.1100	0.0615	0.0660	0.0700	0.0600	0.0560	0.0515	0.0590	0.0540	0.0545	0.0550	0.0805	0.0730	0.0985	0.1255	0.1175	0.1390	0.1185	0.1475	0.1115	0.1110	0.0875
	23	0.0940	0.0915	0.0880	0.0485	0.0655	0.1065	0.1290	0.1195	0.1260	0.1015	0.0905	0.1095	0.1105	0.0850	0.0760	0.0765	0.0735	0.0700	0.0710	0.1475	0.1210	0.1350	0.1540	0.1175	0.1185	0.1260	0.1455	0.1270	0.1375	0.1230
	24	0.0860	0.0850	0.0710	0.0500	0.0805	0.0995	0.1015	0.1080	0.1355	0.1025	0.1105	0.1750	0.1980	0.1140	0.0920	0.1110	0.0800	0.0790	0.0755	0.1390	0.1290	0.1315	0.1825	0.1305	0.1345	0.1480	0.1930	0.1260	0.1430	0.1450
	25	0.0610	0.0550	0.0625	0.0485	0.0545	0.1035	0.0920	0.0985	0.1005	0.0645	0.0730	0.0695	0.1015	0.0910	0.0945	0.0890	0.0805	0.0920	0.0905	0.2115	0.2125	0.2130	0.2290	0.1865	0.1810	0.1945	0.2125	0.1940	0.2010	0.1745
	29	0.0470	0.0520	0.0555	0.0430	0.0390	0.0755	0.0735	0.0955		0.0575	0.0630	0.0595	0.0610	0.0535	0.0545	0.0550	0.0965	0.0605	0.0520	0.1090	0.1025	0.1220	0.1285	0.1150	0.1025	0.1405	0.1625	0.1490	0.1535	0.1560
	31	0.0505	0.0550	0.0530	0.0440	0.0440	0.0650	0.0690	0.0825	0.0905	0.0530	0.0590	0.0685	0.0705	0.0535	0.0520	0.0550	0.0515	0.0570	0.0500	0.0755	0.0675	0.0775	0.1115	0.0725	0.0770	0.0790	0.1170	0.0835	0.0975	0.0970
	32	0.0605	0.0555	0.0500	0.0415	0.0490	0.0600	0.0640	0.0715	0.0860	0.0680	0.0805	0.1200	0.1150	0.0815	0.0645	0.0780	0.0615	0.0710	0.0560	0.0955	0.0810	0.0950	0.1005	0.0765	0.0810	0.0880	0.1245	0.1050	0.1025	0.1090
HW	17	0.0490	0.0480	0.0555	0.0490	0.0440	0.0680	0.0640	0.0850	0.1495	0.0885	0.0885	0.1020	0.0910	0.0695	0.0635	0.0750	0.0650	0.0695	0.0780	0.1400	0.2155	0.2490	0.2425	0.2010	0.1905	0.2495	0.3530	0.2590	0.2845	0.2480
	18	0.0415	0.0435	0.0495	0.0390	0.0370	0.0475	0.0440	0.0600	0.0815	0.0460	0.0540	0.0565	0.0495	0.0465	0.0445	0.0465	0.0445	0.0435	0.0445	0.0535	0.0505	0.0600	0.0655	0.0585	0.0615	0.0560	0.0720	0.0585	0.0665	0.0545
	19	0.0440	0.0470	0.0445	0.0470	0.0420	0.0485	0.0505	0.0620	0.0730	0.0485	0.0560	0.0505	0.0515	0.0465	0.0455	0.0495	0.0470	0.0450	0.0480	0.0525	0.0480	0.0620	0.0675	0.0570	0.0600	0.0585	0.0675	0.0625	0.0620	0.0530
	20	0.0850	0.0680	0.0565	0.0475	0.0475	0.0735	0.0785	0.0880	0.1120	0.0630	0.0735	0.0805	0.0865	0.0660	0.0600	0.0720	0.0610	0.0695	0.0860	0.1590	0.1280	0.1600	0.1635	0.1430	0.1395	0.1380	0.1800	0.1135	0.1185	0.1125
	26	0.0590	0.0620	0.0530	0.0445	0.0485	0.0760	0.0775	0.0940		0.0855	0.0995	0.1495	0.1875	0.1415	0.1460	0.1745	0.1495	0.1365	0.1210	0.2800	0.2550	0.1995	0.2185	0.2305	0.1820	0.2100	0.2220	0.2100	0.1795	0.1620
	27	0.0625	0.0610	0.0605	0.0460	0.0460	0.0695	0.0665	0.0835	0.1050	0.0655	0.0675	0.0660	0.0695	0.0600	0.0535	0.0575	0.0545	0.0525	0.0520	0.0860	0.0830	0.1020	0.0920	0.0755	0.0760	0.0910	0.1015	0.0800	0.0960	0.0775
	28	0.0810	0.0740	0.0625	0.0490	0.0815	0.1015	0.1035	0.1155	0.1230	0.0835	0.0950	0.1410	0.1445	0.1105	0.1110	0.1390	0.0515	0.1055	0.1085	0.1985	0.2070	0.2020	0.2065	0.1260	0.1040	0.1895	0.2265	0.2130	0.2130	0.1705
	30	0.0485	0.0510	0.0495	0.0435	0.0405	0.0625		0.0820		0.0665	0.0705	0.0985	0.1040	0.0745	0.0720	0.0875	0.0720	0.0850	0.0740	0.1440	0.1230	0.1410	0.1385	0.1415	0.1420	0.1420	0.1680	0.1445	0.1455	0.1700

43. Individual animal IgM responses to infection measured by ELISA (OD values) without correction for plate to plate variation, (Gambia study, Chapter 6).

(Missing values indicate where the OD's of duplicate wells differed by more than 20% of the anti-log10 of the larger value.)

Plate No.		32	33	34	27	26	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Day of the study		1	3	5	24	26	29	31	33	36	38	40	43	45	47	50	52	54	57	59	61	64	66	68	71	73	75	78	80	82	85
Group	Bull																														
BN	1	0.6335	0.6780	0.6325	0.4385	0.4855	0.5865	0.5935	0.4755	0.8240	0.7435	0.5900	0.8175	0.9135	0.9880	0.9965		0.8110	0.6840	0.6350	1.2060	1.0500	0.8635	0.6230	0.8430	0.8765	0.8125	0.7065	0.7450	0.6690	0.6260
	6	0.2055	0.2050	0.2025	0.2260	0.3455	0.3140	0.3160	0.2175	0.3785	0.5250	0.5590	0.6110	0.6745	0.7060	0.6930	0.5440	0.5020	0.4290	0.3315	0.7635	0.9205	0.4690	0.3915	0.7730	0.5190	0.6005	0.3270	0.3810	0.3075	0.3540
	7	0.2680	0.2810	0.3025	0.4350	0.3205	0.4895	0.4905	0.3555	0.4920	0.6445	0.4945	0.5050	0.7045	0.7770	0.6920	0.5690	0.4860	0.5035	0.4805	0.7075	0.7530	0.4640	0.4915	0.7365	0.5975	0.6145	0.3985	0.4050	0.3405	0.2655
	8	0.4405	0.4435	0.4215	0.5495			0.5000	0.3610	0.8075	0.7030		0.8210	0.9085	0.9945	0.8715	0.7115	0.5865	0.5235	0.5015	0.9740	1.0295	0.7455	1.1090	1.2670		1.0685	0.8545	0.9435	0.7820	0.8450
	13	0.4080	0.4435	0.4345	0.4610	0.3940	0.4905	0.5050	0.4230	0.9895	0.9270	0.9290	0.7215	0.8210	0.8750	0.7625	0.5610	0.4710	0.4415	0.4870		0.7125	0.4765	0.4105	0.7450	0.7605	0.5495	0.5710	0.6230	0.6810	0.6305
	14			0.6500	0.7340	0.6435	0.9075	0.8560	0.7110	0.7700	0.9755	1.0200		1.1640	1.1815	1.1140	1.0295	0.9160	0.8360	0.8600	1.2375		0.9815	0.7755	1.1070	1.0285	0.8675	0.8065	0.9145	0.7430	0.7725
	15	0.5695	0.5250	0.4380	0.5595	0.4745	0.7140	0.7165	0.5270	0.8980	1.0575	0.9485	1.0300	0.9805	1.0960	1.0635	0.8610	0.6690	0.5865	0.6650	0.8990	0.9965	0.5320	0.4730		0.6215	0.5450	0.5310	0.6090	0.5530	0.5295
	16	0.2925	0.3500	0.3325	0.3510	0.4830	0.4840	0.4335	0.3265	0.6165	0.6800	0.7550	0.8870	0.7800	0.8010	0.6780	0.5090	0.5455	0.6540	0.4655	0.7255	0.7760	0.4775	0.5155	0.7020		0.5765	0.4785	0.5000	0.4495	0.4605
BW	2	0.4740	0.5845	0.5690	0.6735	0.5600	0.7145	0.8280	0.6920	0.9600	0.8805	0.8005	0.8170	0.9165	1.0050	0.8545	0.7885	0.6800	0.7075	0.4760	0.8430	0.7240	0.5125	0.4775	0.8775	0.7005	0.6125	0.5565	0.5840	0.5225	0.5085
	3	0.7090	0.7790	0.7145	0.7095	0.6345	0.7585		0.6890	0.9515	0.8125	0.7715	0.7320	0.8320	0.9275	0.9090	0.8295	0.7635	0.6625	0.6025	0.9265	0.9795	0.6525	0.5680	0.8660	0.8015	0.6815	0.7285	0.8650	0.6855	0.5750
	4	0.3580	0.4480	0.4705	0.5205		0.5890	0.5810	0.5270	0.8340	0.6105	0.6260	0.5790	0.7275	0.8995	0.7750	0.6555	0.6155	0.6025	0.6725	1.1525	1.1345	0.8625	0.7850		0.8595	0.6230	0.6085	0.6430	0.8230	
	5	0.5375	0.6630	0.6200	0.7045	0.6315	0.7690	0.7785	0.6590	0.7325	0.8045	0.8020	0.7260	0.7240	0.8785	0.8135	0.7260	0.7855	0.7830	0.6330	1.2080	1.2970	0.8110	0.7825	1.1210	0.9595	0.9405	0.7445	0.7965	0.6225	1.2385
	9	0.5000	0.5120	0.4640	0.5470		0.6135	0.6575	0.5030	0.7695	0.6590	0.7410	0.6825	0.7780	0.9385	0.7630			0.5095	0.7235	1.3295	1.2130	0.9320	0.8260	0.9785	0.8510	0.6160	0.5335	0.4710	0.4000	0.3950
	10	0.2890	0.2950	0.2860	0.4270	0.3255	0.4310	0.5485	0.3540	0.8250	0.6240	0.6665	0.6455	0.7240	0.7160	0.6600	0.6820	0.5850		0.8905		1.1145	0.6205	0.6760	0.9420	0.8185	0.8070	0.6300	0.6695	0.7080	0.6325
	11	0.4565	0.5530	0.5530	0.5210	0.5225	0.5870	0.6445	0.4920	0.6480	0.6365	0.6515	0.6345	0.6630	0.8140	0.7850	0.6375	0.7125	0.5815	0.6845	0.8420	0.8775	0.5390	0.4505	0.9055	0.6585	0.5340	0.4780	0.6430	0.5560	0.5620
	12	0.4945	0.7205	0.7185	0.7400	0.5630		0.7705	0.5880	0.8185	0.9345	0.8690	0.8395	0.8720	0.8480	0.6885	0.5630	0.4990	0.4135	0.4305	0.7465	0.7395	0.4295	0.7205	0.9925	0.8300	0.6285	0.5330	0.7015	0.5835	0.6355
HN	21	0.2925	0.3265	0.2735	0.3230	0.2830	0.3995	0.3795	0.3575	0.6760	0.5275	0.6940			0.8930	0.8430	0.7855	0.6710	0.6640	0.6485	1.1200	0.9680	0.7255	0.5910	0.9530	0.8225	0.7205	0.5630	0.6870	0.4830	0.3790
	22	0.3270	0.3570	0.3610	0.3555	0.3380	0.4905	0.4940	0.3270	0.5385	0.3910	0.4035	0.6410	0.7595	0.8530	0.7535	0.6630	0.5625	0.4535	0.7055	0.9360	1.1935		0.3780	0.6655	0.5100	0.3470	0.3370	0.4225	0.3645	0.3495
	23	0.3255	0.3990	0.3435	0.4510	0.5450	0.5670	0.5190	0.4375	0.5205	1.0420	0.7835	0.6480	0.6490	0.7170	0.8005	0.8145	0.7580	0.6450	0.6800	1.2095	1.2305	1.0530	1.0100	1.0460	0.7390	0.6365		1.0150	0.8305	0.7100
	24	0.3410	0.4370	0.4050	0.4740	0.4340	0.5930	0.5245	0.3835	0.5525	0.7490	0.5730	0.5770	0.5835	0.5945	0.5930	0.4835	0.4945	0.4295	0.4325	0.7295	0.7410	0.4915	0.4580	0.6875		0.4935	0.5595	0.5965	0.6220	0.4075
	25	0.3065	0.3645	0.3165	0.3795	0.3000	0.4500	0.4460	0.3510	0.3995	0.4620	0.3920	0.4910	0.5230	0.7260	0.7485	0.6970	0.6975		0.4270		0.6905	0.7885	0.4870	0.6475	0.5885	0.4675	0.4465	0.7090	0.4975	0.3625
	29	0.2230	0.3205	0.2880	0.3675	0.2695	0.4250	0.4145	0.3685	0.6055	0.5600	0.5055	0.6330	0.7965	0.9295	0.7730	0.6130	0.4805	0.4365	0.3915	0.8225	0.5345	0.4430			0.5230	0.4190	0.8460	0.6535	0.5370	0.3350
	31	0.3775	0.3395	0.3380	0.3760	0.3010	0.4820	0.4615	0.3855	0.6320	0.8140	0.5665	0.5815	0.5755	0.7125	0.6990	0.5835	0.4675	0.4890	0.4440	0.8175	0.7525	0.4005	0.5860	0.8555	0.9090	0.6080	0.5085	0.4275	0.4250	0.3725
	32	0.2870	0.4425	0.4560	0.4305	0.4685	0.6160	0.5380	0.4460	0.4310	0.6400	0.5220	0.5295	0.6600	0.7735			0.4360	0.3160	0.3660	0.5010	0.4990	0.3895	0.3740	0.7265	0.6290	0.4565	0.3720	0.4745	0.5050	0.4090
HW	17	0.2145	0.2710	0.2535	0.2780	0.2810	0.3395	0.3370	0.3015	0.5535	0.3570	0.3230	0.5050	0.3565	0.4975	0.4335	0.3650	0.3255	0.2775	0.2710	0.7320	0.4775	0.3900	0.2965	0.5615	0.5065	0.3865	0.3540	0.5720	0.6030	0.7730
HW	18	0.3925	0.4390	0.4275	0.4845	0.3970	0.5805	0.6060	0.5045	0.6000		0.6070	0.5645	0.5900		0.5905	0.4870	0.4175	0.3920	0.4195	0.7015	0.5600	0.5765	0.4540	0.7840	0.7335	0.5365	0.4980	0.4730	0.4715	0.3985
HW	19	0.2540	0.3055	0.2935	0.3060	0.2855	0.4140	0.4185	0.3040	0.5345		0.4535	0.4565	0.4520	0.5400	0.4215	0.3390	0.2915	0.3030	0.3050	0.5410	0.4015	0.2875	0.3250	0.3690	0.5040	0.4735		0.4395	0.4125	0.3580
HW	20	0.3270	0.4155	0.4060	0.4165	0.3300	0.4980	0.4320	0.3745	0.6050	0.4895	0.5775	0.7930	0.7440	0.9465	0.8795	0.6360	0.5170	0.7545	0.7330	0.8520	1.0180	0.5050	0.4125	0.5825	0.5830	0.4705	0.5065	0.4970	0.5345	0.4725
HW	26	0.2280	0.2845	0.2440	0.3145	0.2920	0.4770	0.3875	0.3490	0.6715	0.9100	0.6715	0.7355	0.6035	0.6395	0.6795	0.5475	0.4815	0.4225	0.3265	0.6830	0.6960	0.3375	0.3185	0.7120	0.6060	0.5320	0.4535	0.5495	0.4275	0.4155
HW	27	0.2980	0.4210	0.4095	0.3760	0.2610		0.4270	0.3570	0.5115	0.5805	0.5400	0.5895	0.5390	0.5940	0.6890	0.5565	0.4295	0.3165	0.3250	0.5830	0.4590	0.3335	0.3400	0.6675	0.6445	0.5850	1.0330	0.9340	0.7145	0.4655
HW	28	0.8660	0.8620	0.8405	0.6335	0.4440	0.7350	0.6710	0.4975	0.5550	0.4785	0.5835	0.8455	0.8980	0.9160	0.9195	0.7800	0.6510	0.7325	0.9975			1.0000	0.8920	1.2230	1.1100	0.8195	0.8665	0.9615	0.8485	0.7500
HW	30	0.4805	0.5380	0.6890	0.6175	0.5620	0.7305	0.7955	0.6675	0.7465	0.7840	0.6865	0.6875	0.7060	0.8235	0.7140	0.4935	0.4890	0.4335	0.5420	1.2865	1.4645	0.7030	0.5030	0.8935	0.7540	0.7630	0.8995	0.7480	0.7030	0.8050

44. Individual animal total IgG responses to infection measured by ELISA (OD values) without correction for plate to plate variation, (Gambia study, Chapter 6).

(Missing values indicate where the OD's of duplicate wells differed by more than 20% of the anti-log10 of the larger value.)

Plate No.		34	35	36	37	33	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Day of the study		1	3	5	24	26	29	31	33	36	38	40	43	45	47	50	52	54	57	59	61	64	66	68	71	73	75	78	80	82	85
Group	Bull																														
BN	1	0.8205	0.7980	0.7505	0.1285	0.1895	0.1775	0.1395	0.1440	0.2040	0.2090	0.1965	0.2190	0.2005	0.2360	0.2450	0.2825	0.3210	0.3485	0.2630	0.2480	0.2895	0.1645	0.2275	0.4225	0.4340	0.4745	0.6055	0.4580		0.5135
	6	0.1775		0.1800	0.1175	0.1670	0.1220	0.0985	0.0965	0.1630	0.2345		0.2330	0.2145	0.2460	0.3000	0.2690	0.3180	0.2545	0.2315	0.2200	0.2220	0.1310	0.1610	0.3110	0.3265	0.3605	0.4145	0.3540	0.3840	0.3695
	7	0.2100	0.1815	0.1740	0.1075	0.1870	0.1395	0.1280	0.1105	0.1380	0.1640	0.1525	0.1695	0.1675	0.1895	0.2270	0.2115	0.2175	0.2310	0.2140	0.1785	0.2470	0.1115	0.1555	0.3090	0.2930	0.3210	0.3515	0.2995	0.3045	0.2965
	8	0.2375	0.2215	0.2115	0.1645	0.2010	0.2015	0.1660	0.1660	0.2030	0.1915	0.2165	0.2325	0.2180	0.2335	0.3185	0.3185	0.2995	0.3000	0.3785	0.3205	0.4270	0.1810	0.3160		0.5620	0.5380	0.5865	0.6060	0.6245	0.5975
	13	0.2245	0.2260	0.2005	0.1380	0.1800	0.1635	0.1380	0.1370		0.2050	0.2240	0.2020	0.2190	0.2055		0.2440	0.2265	0.2060	0.1935	0.1650	0.1885	0.1235	0.1325	0.2795	0.2825	0.2600	0.3075	0.2870	0.2765	0.2510
	14	0.2080	0.1920	0.2000	0.1120	0.1900	0.1680	0.1370	0.1135	0.1290	0.1565	0.2040	0.2250	0.2245	0.2275	0.3335	0.2715	0.2735	0.2465	0.2410	0.2280	0.2365	0.1470	0.1700		0.3265	0.3110	0.3380	0.3160	0.2945	0.3005
	15	0.2270	0.2205	0.2305	0.1525	0.2205	0.2300	0.1595	0.1620	0.1935	0.2830	0.2880	0.3330	0.2760	0.2540	0.4590	0.3865	0.3420	0.3655	0.3120	0.2795		0.1805	0.2470		0.4035	0.3945	0.5115	0.4220	0.3960	0.3575
	16	0.1965	0.2055	0.1855	0.0995	0.1810	0.1615	0.1315	0.1160		0.1170	0.1755	0.1805	0.1560	0.1445	0.1925	0.1760	0.1865	0.1740	0.1460	0.1405		0.0995	0.1335	0.2590	0.2885	0.2875	0.3215	0.3255	0.3090	0.3075
BW	2	0.3180	0.3395	0.3370	0.2885	0.3120	0.2435	0.1980	0.2385	0.2925	0.2610	0.2790	0.2625	0.2625	0.3300	0.3360	0.3610	0.3565	0.3550	0.2930	0.2885	0.2930	0.1760	0.2140	0.4120	0.4445	0.4375	0.4830	0.4620	0.4630	0.3935
	3	0.2120	0.2170	0.2010	0.1265	0.1970	0.1540	0.1300		0.1860	0.1710	0.1720	0.2290	0.1855	0.2355	0.2815	0.3100	0.3105	0.3155	0.2540	0.2260	0.3100	0.1430	0.2270	0.4230	0.4345	0.4295		0.4525	0.4700	0.4665
	4		0.1815	0.1660	0.1090	0.1680	0.1865	0.1385	0.1200	0.1945	0.1880	0.2500	0.2225	0.1745	0.1895	0.2205	0.2100	0.2020	0.2370	0.2320	0.2060		0.1100	0.2250	0.4490	0.4185		0.5225	0.4550	0.4490	0.3680
	5	0.3410		0.3365	0.2555	0.3085	0.3575	0.3330	0.2485	0.2190	0.2785	0.3195	0.3245	0.2955	0.2820	0.2690	0.2875	0.3020	0.3475	0.2735	0.2830	0.3085	0.1690	0.1955	0.3400	0.4125	0.4140	0.4830	0.4050	0.3880	
	9	0.2365	0.2115	0.2050	0.1365	0.1855	0.1710	0.1495	0.1420	0.1870	0.1885	0.2035	0.2045	0.2060	0.2240	0.2625	0.2595	0.2155	0.2150	0.2355	0.2100	0.2555	0.1405	0.1550	0.3405	0.3130	0.3345	0.3605	0.3230	0.3125	0.3145
	10	0.2240	0.2065	0.2005	0.1500	0.2115	0.1770	0.1710		0.2330	0.2050	0.2340	0.2480	0.2280	0.2315	0.3355	0.3240	0.3340	0.4335	0.4395	0.4120	0.3985	0.2625	0.3000	0.6005	0.6170	0.6285	0.6930	0.6380	0.7175	0.6270
	11	0.1960	0.1930	0.1850		0.1910	0.1445	0.1200	0.1180	0.1530	0.1755	0.1975	0.1995	0.1355	0.1690	0.2090	0.2150	0.2255	0.2320	0.2255	0.1930	0.2035	0.1205	0.1465		0.3255	0.4035	0.5190	0.5005	0.4940	0.4725
	12	0.1710	0.1895	0.1825		0.1740	0.1650	0.1285	0.1090	0.1495	0.1290		0.1450	0.1215	0.1275	0.1375	0.1445	0.1320	0.1415	0.1180	0.1255	0.1910	0.0650	0.1150	0.2840	0.2795	0.2680	0.3050	0.3125	0.3135	0.3390
HN	21	0.2100	0.2050	0.1960	0.1220		0.1595	0.1315	0.1355	0.1870	0.1895	0.2990	0.3635	0.3020	0.2665	0.3325	0.3470	0.3110	0.4185	0.2510	0.2985	0.2875	0.1980	0.1830	0.4145	0.3685	0.3935	0.3870	0.3480	0.3825	0.3745
	22	0.1825	0.1730	0.1805	0.0985	0.1505	0.1160	0.1170	0.1100	0.1235	0.1160	0.1300	0.1400	0.1515	0.1600	0.2185	0.2205	0.2150	0.2105	0.1955	0.1960	0.1740	0.1315	0.1430	0.3130	0.3310	0.3085	0.3810	0.3520	0.3635	0.3325
	23	0.2245	0.2105	0.2125	0.1520		0.1890	0.1695	0.1480	0.1610	0.2370	0.2325	0.2645	0.1820	0.1985	0.2915	0.2615	0.2680	0.2690	0.2225	0.2395	0.2795	0.1675	0.2090	0.3695	0.3020	0.3260	0.3390	0.3295	0.3750	0.3330
	24	0.2065	0.2065	0.1975	0.1210	0.1735	0.1570	0.1515	0.1540	0.1510	0.2000	0.2160	0.3490	0.2090	0.1945	0.2970	0.2755	0.2400	0.2660	0.2470	0.1975	0.2680	0.1305	0.1785	0.3655	0.3370	0.3175	0.3640	0.3235	0.3340	0.3130
	25	0.1745	0.1700	0.1770	0.1200	0.1810		0.1175	0.1270	0.1155	0.1610		0.1460	0.1510	0.2030	0.3105	0.2715	0.2580		0.2005	0.2265	0.2720	0.2410	0.1880	0.3675	0.3435	0.3615	0.3945	0.3455	0.3880	0.3375
	29	0.1925	0.2160	0.1930	0.1145	0.1790	0.1540	0.1365	0.1375	0.1710	0.1725	0.1810	0.2000	0.2040	0.1960	0.2555	0.2765	0.2430		0.2110	0.1940	0.2755	0.2345	0.1875	0.4005	0.3965	0.3850	0.4805	0.4090	0.4765	0.4405
	31	0.1825	0.1820	0.1880		0.1615	0.1490	0.1435	0.1340	0.1420	0.1945	0.1900		0.1615		0.2110	0.1950	0.2035	0.1910	0.1855	0.1525	0.1815	0.0825	0.2105	0.3145	0.2900	0.2720	0.3080	0.2735	0.2870	0.2510
	32	0.2095	0.1745	0.1860	0.0905	0.1525	0.1415	0.1245	0.1050	0.1205	0.1295	0.1760		0.1875	0.1840	0.2590	0.2290	0.2510	0.1935		0.1535	0.2250	0.0730	0.1740	0.2885	0.2625	0.3040	0.3350	0.2975	0.3125	0.3015
HW	17	0.1810	0.1820	0.1935	0.1090	0.1755	0.1595	0.1190	0.1355	0.2240	0.2400		0.2435	0.2035	0.2150		0.2760	0.2485	0.3230	0.2620	0.2675	0.3350	0.2530	0.2645	0.4845		0.5550	0.6565	0.5565	0.6210	
	18	0.1850	0.1730	0.1740	0.1030	0.1645	0.1445	0.1185	0.1320	0.1220	0.1705	0.1685	0.1230	0.1210	0.1215	0.1615	0.1695	0.1525	0.1750	0.1885	0.1320	0.1655	0.1040	0.1275	0.2625	0.2720	0.2575	0.3095	0.2740	0.2890	0.2765
	19	0.1655	0.1600	0.1685	0.0905	0.1660	0.1320	0.1095	0.1095	0.1245	0.1305	0.1440	0.1300	0.1325	0.1220	0.2130	0.1865	0.2075	0.1765	0.1480	0.1285	0.1905	0.0830	0.1355	0.3045	0.2875	0.2870	0.3105	0.3075	0.2985	0.2795
	20	0.1955	0.1820		0.1205	0.1935	0.1835	0.1600	0.1475	0.1925	0.1740			0.2165	0.2500	0.3135	0.3130	0.3400	0.3635	0.3275		0.4100	0.1895	0.2560		0.4795	0.4300	0.4930	0.4875	0.4365	0.3865
	26	0.2295	0.2285	0.2115	0.1180	0.1830	0.1645	0.1420	0.1375	0.1725	0.2120	0.2360	0.2935	0.2330	0.2620	0.3610	0.3730	0.3280	0.2780	0.3110	0.2855	0.2545	0.1620	0.2240	0.3780	0.3870	0.3795	0.3735	0.3565	0.3670	0.3685
	27	0.1745	0.1960	0.1765		0.1740	0.1480	0.1310	0.1140	0.1465	0.1480	0.1575	0.1735	0.1235	0.1155	0.1730	0.1750	0.1635	0.1605	0.1580	0.1335	0.1985	0.1090	0.1455	0.2305	0.2295	0.2530	0.3185	0.2930	0.2710	0.2160
	28	0.2505	0.2370	0.2640	0.1480	0.2000	0.2230	0.1980	0.1735	0.1700	0.1555	0.2490	0.4630	0.3070	0.3060	0.4350	0.4180	0.4295		0.4405	0.3200		0.1650	0.2710	0.4400	0.3850	0.4125		0.4840	0.4570	0.3630
	30	0.1890	0.1875	0.1855	0.1395	0.1775	0.1590	0.1545	0.1465	0.2108	0.1915	0.1990	0.2105	0.2200	0.2160		0.2950	0.3015	0.2935	0.2575	0.3040	0.2540		0.1855	0.4455	0.3845	0.4100	0.5265	0.4345	0.4395	0.4595

45. Dotblot methodology used to test the reactivity of the mouse anti-bovine IgG_2 monoclonal antibody from the IgG_2 ELISA.

A piece of polyvinylidene difluoride (PVDF) membrane was marked out in a six by six grid of one centimetre squares. Two microlitres of an IgG_2 enhanced serum (Serotec Ltd.) containing 25 mg of IgG_2 /l was spotted into each square in two of the six columns. Two further columns were filled with dots of the serum diluted 1:10 with PBS-Tween (2.5 mg of IgG_2 /l). The remaining two columns were filled with dots of the ELISA conjugate (goat anti-mouse IgG (whole molecule) horse radish peroxidase), in one column neat in the other diluted 1:1000 with PBS-Tween. The dots were left to soak into the membrane and dry before being fixed in a bath of methanol. Thereafter the membrane was washed overnight in PBS-Tween on a plate shaker.

The following morning the PBS-Tween was drained off and the membrane was flooded with the mouse anti-bovine IgG_2 monoclonal antibody (Serotec Ltd.) diluted 1:50 in PBS-Tween. After a further 30 minutes on the plate shaker the monoclonal antibody was discarded and the membrane washed three times in PBS-T solution (pH 7.4) with a soak period of 3 minutes between washes. It was then flooded with goat anti-mouse IgG (whole molecule) horseradish peroxidase conjugate (Sigma Chemical Co. Ltd.) diluted 1:1000 with PBS-Tween and shaken for a further 30 minutes, before being washed three times as before.

A 4 -chloro-1-naphthol peroxidase substrate was made up by dissolving 60 mg of 4 -chloro-1-naphthol in 20 mls of ice cold (–20°C) methanol, the resultant solution being kept chilled and out of the light. Immediately before the substrate was required, 60µl of cold (4°C) hydrogen peroxide was added to 100 mls of TRIS buffered saline and then mixed with the naphthol solution to produce the substrate solution, which was then poured onto the membrane. The membrane was shaken for one hour at room temperature before the substrate was poured off. The reaction was stopped by neutralising the medium with distilled water.

The membrane was visually examined. Dots in the columns which contained the class specific monoclonal antibody indicated that the monoclonal was reacting effectively.

46. List of equipment & reagent suppliers.

BECTON DICKINSON LTD.
Between Townsroad, Cowley, Oxford, OX4 3LY, UK

BINDING SITE LTD.
Institute of Research & Development, Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, UK

BIOFUGE A HERAEUS SEPATECH
(address not known)

BOOTS LTD.
Nottingham, UK

CIBA GEIGY AGRICULTURE LTD.
Whittlesford, Cambridge, CB2 4QT, UK

DYNATECH LABORATORIES LTD.
Daux Rd. Billingshurst, Sussex, RH14 8SJ, UK

ETABLISSEMENTS AKRA
11 Avenue Edouard VII, 64000 Pau, FRANCE

FISSONS INSTRUMENTS LTD.
Sussex Manor Park, Gatwick Rd. Crawley, Sussex, RH10 2QQ, UK

HAWKSLEY & SONS LTD.
Lancing, UK

HOECHST UK LTD.
Walton Manor, Walton, Milton Keynes, UK.

HUSKY MICROCOMPUTERS LTD.
PO Box 135, 345 Foleshill Rd. Coventry, CV6 5RW, UK

JENCONS SCIENTIFIC LTD.
Cherrycourt Way Industrial Estate, Stanbridge Rd., Leighton Buzzard, LU7 8UA, UK

LAB SYSTEMS LTD.
Unit 5, The Ringway Centre, Edison Rd., Basingstoke, Hants, RG21 2YH, UK

LIFE TECHNOLOGIES LTD.
3 Fountain Drive, Inchinnan Business Park, Paisley, PA4 9RF, UK

MERCK SHARP & DOHME LTD.
Hertford Rd., Hoddesdon, Hertfordshire, EN11 9BU, UK

MICROSOFT CORPORATION INC.
Microsoft Place, Winersh, Wokingham, Berkshire, RG41 5TP, UK

MINITAB INC.
3081 Enterprise Drive, State College, PA 16801-3008, USA

NOVATECH MEASUREMENTS LTD.
83 Castle Ham Rd. St. Leonards on Sea, Sussex, TN38 9NT, UK

NUMERICAL ALGORITHMS GROUP LTD.
Oxford, UK

PITMAN-MOORE LTD.
Crewe Hall, Crewe, Cheshire, CW1 1YR, UK

RHONE MERIEUX LTD.
Spire Green Centre, Harlow, Essex, CM19 5TS, UK

RITCHIE FARM EQUIPMENT LTD.
Forfar, Scotland.

ROCHE PRODUCTS LTD.
Welwyn, UK

SEAFIELD MILL LTD.
by Bilston, Roslin, Midlothian, EH 25 9RQ

SEROTEC LTD.
22 Bankside, Station Approach, Kidlington, Oxford, OX5 1JE, UK

SIGMA CHEMICAL CO. LTD.
Fancy Rd., Poole, Dorset, BH17 7NH, UK

TRUMETER LTD.
Manchester, UK

TRU-TEST DISTRIBUTORS LTD.
24 Rakau Drive, PO Box 51-078, Pakuranga, Auckland, New Zealand

WHATMAN INTERNATIONAL LTD.
20/20 St. Leonards Rd., Maidstone, Kent, ME16 0LS, UK